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ON THE CYTOLOGY AND LIFE HISTORY OF THE AMOEBAE*

WITH DESCRIPTIONS OF TWO NEW SPECIES

By R. W. Glaser

INTRODUCTION

It was shown by Graybill and Smith (1920) and Smith and Graybill (1920) that blackhead in turkeys and chickens could be experimentally produced by feeding the embryonated eggs of the nematode, *Heterakis papillosa*. They had frequently encountered protozoa in the cultures in which the ova were developing, and it was considered advisable to determine whether or not these organisms represented some stage of the protozoon responsible for the disease. These results led the writer to make a careful study of the protozoan fauna encountered in Heterakis, as well as in the embryonated egg cultures. These investigations disclosed nothing of importance concerning blackhead, but the amoebae studied revealed a number of new and interesting cytological and life history characters.

The best method for obtaining the amoebae under discussion consists in removing adult Heterakis from the ceca of a variety of healthy chickens. The worms are thoroughly washed in sterile physiological salt solution, and sedimented several times. They are then cut up and the material incubated in physiological salt solution for fifteen to thirty days at room temperature. During the summer of 1920, nineteen cultures were thus prepared from different lots of chickens.

On examining incubated Heterakis cultures in from two to three weeks, it was found that besides worm fragments, embryonated Heterakis ova, and bacteria, the cultures nearly always contained the trophozoites and cysts of amoebae. Two species were found in the salt solution cultures either together in the same culture or separately in different cultures. The amoebae were easily recognized, particularly the cysts resting on the bottom of the Petri dish, but when the writer was in doubt, he placed a worm fragment under the microscope. Such a fragment usually proved to contain many amoebae which probably

* From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

fed upon the countless bacteria found within the dead worm tissues. The two forms were the only amoebae which developed within the salt solution cultures. By the use of the methods of Frosch (1897) and Walker (1908), the amoebae were isolated and grown on Musgrave and Clegg's medium, 0.7 of normal alkaline, in association with cultures of *Staphylococcus pyogenes albus*.

For the study of some of the stages the writer found Walker's hanging plate method indispensable, but found and Walker also noted that the amobae will usually not pass through their complete life cycle on the hanging plate but take the short cut from cyst to cyst merely through the trophozoite stage. In order to obtain all of the stages, one must also resort to the Petri plate cultures.

The various stages that were fixed and stained were always compared with the living material of similar stages that grew on the hanging plate cultures. One can obtain a much more accurate picture of the organisms from the hanging plate cultures than by removing some of the organisms from Petri plate cultures to some liquid medium like Ringer's, Locke's, or physiological sodium chloride solution. Should the latter procedure be necessary, one must be careful to use a solution strictly isotonic to the liquid constituents of the protoplasm, otherwise one produces certain deformations of the organisms, manifested by plasmolysis or extensive grosser granulations and other cytologic structural changes that do not normally exist.

When certain stages fail to develop on the hanging plate, it is necessary to consult the Petri plate cultures and examine the organisms in some carefully balanced non-toxic solution, but the best results are obtained by removing the stages from the Petri culture and by immersing them immediately in some fixative. Fixation was accomplished either by means of Zenker's or Schaudinn's solutions. Mallory's chloride of iron hematoxylin was used extensively after Zenker's fixation, but some stages of the amoebae were better elucidated by means of Delafield's or Heidenhain's iron hematoxylin after Schaudinn's corrosive sublimate alcohol mixture.

Vital staining (methylene blue and especially neutral red 1 : 3,000 to 1 : 10,000) was used during the studies on cysts and the method of sporulation. This method cleared up much that remained obscure when fixing and other staining methods were used.

It seems advisable to tentatively place the two organisms studied by the writer in the genus Amoeba rather than in the genus Entamoeba for reasons which will become apparent later. However, it might be well to state that no evidence was found to support the view that the organisms to be described multiply or pass through any stages within the intestines or ceca of chickens. Recently Tyzzer (1920) described two true host inhabiting forms in chickens and turkeys. He did not

report stages of the organisms described in this article, so it seems probable that the amoebae here described are what are known as intestinal passers, possibly derived from the soil, and remain in the encysted stage until eliminated with the excrement.

Amoeba commutabilis sp. nov.

This amoeba when cultivated on the hanging plates (sealed depression slide cultures) usually presents only the simple amoeboid and cyst stages. If the medium on the cover slip is inoculated with cysts and some food bacteria *(Staph. pyogenes albus)*, the trophozoites appear in 48 hours at room temperature or sooner at 32° C. The trophozoites are exceedingly sluggish. The cytoplasm is clear, faint and finely granular. There is no distinct differentiation between endo- and ectoplasm except when the pseudopodia form. The formation of the pseudopodia is eruptive, and when they erupt they often run along the periphery of the amoeba in a wave-like motion. There seems to be no pronounced flowing of the endoplasm. Sometimes the pseudopodial processes are quite pointed, but usually blunt. They are always rather short. A few food vacuoles are present containing bacteria during the feeding phase. A small contractile vacuole is present. The nucleus is clear and faint with the chromatin concentrated within the karyosome during the feeding and growing phase. Plate I, figs. 1, 2, 4 and 5 represent living amoebae of the growing and feeding phase. Figures 1 and 2 represent amoebae examined in isotonic solution and showing no clear differentiation between endo- and ectoplasm. The ectoplasm in these cases appears granular. Figures 4 and 5 represent amoebae examined on depression slide cultures showing differentiation between endoplasm and pseudopodial ectoplasm. When conditions become unfavorable on the depression slides, the amoebae first become quiescent, then free themselves of waste products, assume a round form and encyst (Fig. 6). Encystment on the depression slides occurs in four or five days. The early cysts still show the chromatin concentration represented by the karyosome which is surrounded by a clear, distinct, but chromatin free nucleus. The cysts show only one wall when examined on the depression slides or in a strictly isotonic solution in which no plasmolysis occurs. The resting trophozoites measure 5 to 8μ. The cysts measure 6μ.

The agamic multiplication of the trophozoites can best be followed by roughly detecting the nuclear and karyosomic changes on the depression slide, and by immediately removing the cover slip containing the agar film culture, immersing in the fixative, and staining carefully by one of the histologic methods outlined previously.

Neither a sexual phenomenon nor the suggestion of one was encountered. The trophozoites multiply by a sort of primitive mitosis known as promitosis, or the division of the karyosome. Hartmann did

much work on this method of cell division, but certain important details found in this amoeba seem to differ from methods heretofore described. In the most primitive protozoa showing this form of nuclear division, Hartmann found that the karyosome contains all the chromatin and nuclear material and is submerged in a zone of nuclear sap. No definite nuclear membrane exists. In higher forms chromatin and supportive structures are found within the nucleus proper and a definite nuclear membrane appears.

During the course of the studies on *A. commutabilis,* it was found that at certain times the amoebae could be placed within the primitive group and at times within the modified group. At a certain stage the karyosome contains all the chromatin and is situated in clear nuclear sap containing no supportive structures. At another stage the karyosome gives off chromatic material (chromidia) into the nucleus or the karyosome may disappear entirely, all the chromatic material being contained within a large diffuse nucleus. In still other stages the nucleus disappears, the chromatic material diffusing out into the cytoplasm, and later, at another stage, to be reorganized and incorporated into spore material. The presence of a nuclear membrane is never much accentuated at any stage.

The changes observed by the writer and outlined above have been attributed to physiologic cyclic changes and, in this case at least, he is unable to decide upon the phylogenetic status of the organism based on the structure of the nucleus and karyosome.

The trophozoites preparatory to division come to rest and assume a more or less round form (prophase). The karyosome then splits into two equal halves (Pl. I, Figs. 7, 8, metaphase). A centriole was never seen. In the next stage or early anaphase (Fig. 9) the nucleus is greatly hypertrophied and contains a spindle with the karyosomic halves diverging. In a late anaphase (Fig. 10) the nucleus has disappeared; an interzonal region shows clearly between the two karyosomic halves and there is a weak suggestion of a modification of the astral ray phenomenon. Figure 11 represents the telophase with the organization of two nuclei containing each a karyosome. This condition is later followed by cell division into two daughter amoebae which separate and move off one from another. The agamic method of reproduction in this amoeba seems not to be preceded or accompanied by any phenomenon of reduction. At this time no purification of the karyosome occurs as recognized by the formation of chromidia in the nucleus. The nucleus during its existence up to the late anaphase remains perfectly clear.

Another method of multiplication is resorted to in this species, namely, through the formation of spore-like structures homologous to those found by Schaudinn in *Entamoeba histolytica,* and by Walker in

a number of amoebae parasitic within the intestinal tract. The details of this sporulation process in *A. commutabilis* again differ from the previously described cases. Sporulation occurs rarely on the depression slides (hanging plates), but most frequently in the Petri plate cultures. Indeed, even in such cultures very often the cyst, trophozoite cyst cycle may be all that is revealed and the other stages are entirely omitted. The conditions under which these phenomena occur or not are obscure. Physical factors may be involved or certain processes may occur only after a definite number of agamic reproductive generations. Sporulation, when it does occur within a Petri plate culture, is quite universal within the particular culture, and one has no difficulty in tracing certain details of the process and some of the interesting ones that follow.

As was pointed out previously, the early cyst assumes the aspect illustrated on Plate I, Fig. 6. The amoeba assumes a round form, with the chromatic material concentrated as a karyosome and submerged within a clear nucleus. The time required for certain changes varies so much that it is impossible to give any specific data on this point. Generally speaking, in about 12 hours the karyosome begins to purify itself by throwing out or secreting chromidia into the nucleus (Fig. 12). On account of the minuteness of the structures involved in the process, it is impossible to affirm with any conviction that the chromidia are ultimately incorporated within the forming spores and that later some of them are also used during the synthesis of the new trophozoite nucleus. However, the process as it was observed is very suggestive of these theoretical assumptions.

About 6 hours after the stage described above the condition represented by fig. 13 is found. The karyosome has vanished or has been entirely resolved into chromidia, which are now represented collectively by a large diffuse nucleus. Still later (Fig. 14) the nucleus disappears and minute, deeply staining chromatic bodies are found scattered through the cytoplasm. Later stages (Figs. 13 to 18) follow rapidly; some of them seem to consume only a few hours. Figure 15 shows the growth of the deeply staining bodies which are the so-colled spores. If this stage is transplanted to fresh media containing living bacteria, the trophozoite emerges from the cyst in about 12 hours. From cyst to trophozoite, when a fresh cyst is transplanted, consumes in all about 48 hours. The newly emerged trophozoite still contains no nucleus; only spores varying in numbers from five to eight (Fig. 16). The spores also vary considerably in size from quite small ones to some approaching the size of the karyosome of this species. At a later stage (Fig. 17) the trophozoite shows a nucleus and karyosome. The spores are now extruded within a few hours. At this period the writer found no large amoebae undergoing cytolysis, so it appears that the adults after spore extrusion continue to live and divide agamically.

The method of sporulation described does not harmonize with Walker's (1908) general statement that "sporulation like other vital processes, ceases with the encystment of the amoebae." Also in no case was Walker able to observe any nuclear changes preceding or during the process of sporulation. Nuclear changes do precede and accompany sporulation in the species here studied, and in this fact we seem to agree more nearly with Schaudinn (1903) who found in the case of *Entamoeba coli* that profound nuclear changes and a sort of sexual process resulting in eight daughter nuclei and in the corresponding number of amoebae accompany the process of encystment. It seems to be unsafe to make a general statement covering all of the manifold and complicated processes occurring within a class of organisms represented by so many species and still so little understood. One set of cytological and other phenomena may be observed in one species, whereas the reverse or a totally different set of processes may become apparent in another.

In *A. commutabilis* the spores soon develop on the Petri plate cultures if conditions are favorable. Often they fail to develop at all, and it is necessary to make many cultures in order to procure a number that are favorable for study. Development seems to be direct (Fig. 18) as Walker found in other species. At first the spore resembles the karyosome of the amoeba in size and structure. Many individuals show a densely staining periphery and a lighter center. In a short time, the spore enlarges and becomes delicately and uniformly granular throughout with a small number of deeply staining granules in the cytoplasm. The next stage assumes a more amoeboid aspect; larger in size with the organization of chromatic material near the center. The final stage in this series is represented by a small amoeba with a well organized nucleus and karyosome. At this time the organism shows amoeboid movement.

The possibility exists that the deeply staining bodies encountered and termed spores may not be spores at all, but deeply staining chromatic material with a small enveloping layer of cytoplasm which the writer was unable to demonstrate due to faulty technic. In comparison with certain cytologic changes occurring in *E. coli* and *E. histolytica,* it is conceivable that the deeply staining bodies really represent aggregations of chromidia which later surround themselves with a small amount of cytoplasm. These are extruded as very minute amoebae, at first showing no amoeboid movement. The parent *A. commutabilis* does not fragment; the young amoebae or spores are extruded. No autogamous processes preceding the formation of these so-called spores was observed.

Another stage in the complicated life cycle of this amoeba was found, and this stage was the one which really attracted the writer to

the interesting potentialities of this species. Budding occurs in a large number of cultures under the proper conditions. Agamic simple and multiple budding was observed by Schaudinn in *Entamoeba histolytica*. Schaudinn not only observed this phenomenon in material taken from the intestinal lumen of cases of dysentery, but also observed it between the cells of the intestinal epithelium. Schaudinn considered that amitotic division of the nucleus accompanies this method of multiplication.

In so far as it is possible to determine, Schaudinn seems to have been the only worker who observed budding in amoebae, and when this condition first appeared on the *A. commutabilis* plates a contamination with a saccharomycete or with the yeast-like stages of some of the higher fungi suggested itself. After repeated isolations and platings the writer was able to convince himself that budding occurs at times within the species studied.

Budding when it does occur in *A. commutabilis* appears about 12 to 24 hours after the so-called spores have given rise to small amoebae. The small amoebae at first grow a bit and divide a number of times (Fig. 19). Soon after one begins to recognize such forms as are represented by figure 3 (from living specimen) and figure 20 (fixed and stained). A small bud will be found beginning to constrict off from the parent cell and without the suggestion of any nucleus. The nucleus of the parent cell shows the presence of a karyosome and chromatin granules within the otherwise clear nucleus. These chromatin granules or chromidia undoubtedly diffuse out into the cytoplasm and some of them probably reach the bud before constriction occurs. Figure 21 shows another case of budding with the karyosome in a curious, stellate, cyclic phase. Figure 22 represents a larger amoeba budding and also showing pseudopodial activity. By examining a number of budding cultures in the fresh condition with the aid of the warm stage, the writer has frequently observed budding accompanied by slight and slow pseudopodial activity in certain larger individuals. Generally speaking, however, budding is a function of the small amoebae and usually disappears entirely when they become large. Figure 23 is difficult to interpret. This case may represent unequal division of a small amoeba or rather equal budding or, lastly, it may represent an abnormal condition. Multiple budding, as observed by Schaudinn in *E. histolytica*, does not occur.

After the buds have formed they are gradually constricted off. The time required for this and some of the other processes has not been noted, for the reason that budding does not occur on the hanging plate cultures which lend themselves better to time observations. Figure 24 represents a number of stages in the development of the bud to the small amoeba. The first stage looks much like the adhering bud. It is more or less round and finely granular with no nucleus. Later an

organization of chromatic material occurs and the bud begins to assume an amoeboid form. On examining the Petri cultures still later, one finds typical small amoebae (Fig. 25) showing a clear nucleus with the deeply staining karyosome.

The stages represented by figures 26 and 27 are also difficult of interpretation and I hesitate to offer one. These stages were rarely found in resting amoebae, not cysts, and probably represent nuclear cyclic changes. The ray-like structure (Fig. 26) is not the opening in a cyst through which an amoeba has emerged, but a nucleus with peripheral chromatin and sending chromatic rays into the cytoplasm.

Amoeba vegetabilis sp. nov.

This species is an exceedingly active form when examined on the hanging plate cultures. A distinct differentiation between endo and ectoplasm exists (Plate 11, Figs. 28, 29, 30 and 31) except when in the resting condition (Figs. 35 and 36). A continual streaming of the entire protoplasm occurs. The endoplasm is granular and contains many ingested bacteria. The formation of the pseudopodia is not eruptive as in the preceding species, but they form in an even flowing manner. One or two broad pseudopodia occur which may terminate bluntly (Fig. 28) or in a pointed or saw-toothed fashion (Figs. 29 and 30). A contractile vacuole is present, and an interval of approximately 65 seconds intervenes between two pulsations. After contraction the new vacuole arises again at two or three minute points that increase in size and suddenly flow together into one large vacuole which soon contracts. The nucleus is of the karyosomic type with all the chromatin concentrated in the center and submerged in a clear surrounding area. This type of nucleus seems to exist except during the greatest feeding and digestive phases when (Figs. 32 and 33) the chromatic material is diffuse throughout and no karyosome is present. No especial study of the agamic multiplication of this amoeba was made, but since the nucleus is of the karyosomic type a form of mitosis seems probable.

This amoeba was also studied on Petri plate cultures and the vegetative or agamic method of reproduction appeared to be the only one. The formation of spores or a process of budding was never observed. The cyst, trophozoite, cyst cycle, consuming from 5 to 9 days, constituted the entire history on the media used. When cysts are placed on fresh media in the presence of suitable bacteria (*Staphylococcus pyogenes albus*) and kept at room temperature, the trophozoites emerge in from 24 to 48 hours.

When fixed and stained properly, the cytoplasm of the trophozoites appears to be highly vacuolated (Figs. 32, 33, 34 and 35). Large food vacuoles are also in evidence containing phagocytized bacteria. Figure 33 represents the only fixed specimen found showing a large ectoplasmic pseudopodium. Figure 35 represents a round resting stage.

When the amoeba begins to encyst, it comes to rest, becomes round and the cytoplasm loses its vacuolated appearance becoming granular (Fig. 36). No karyosome can be seen, the entire nucleus staining. After about 12 hours or more the amoeba becomes polygonal or irregular, a contraction of the protoplasm occurs and a two walled cyst is formed (Figs. 37, 38 and 39). In fully formed cysts the amoeba proper may appear to be polygonal and rather regular (Figs. 37 and 38) or it may assume a stellate or irregular form with rounded, blunt angles (Fig. 39). At times the encysted amoeba follows closely the outline of the cyst wall proper, which is usually quite irregularly scalloped. The angles of the amoeba do not touch the cyst wall. The cyst wall proper is clear and hyaline. After the emergence of the amoeba the empty cysts appear yellow in color. One nucleus is present within the cyst. The average resting *A. vegetabilis* measures 10 to 13μ. The nucleus measures 2μ and the cysts 10μ.

The amoeba described as *A. vegetabilis* somewhat resembles Walker's *A. intestinalis*. Several diagnostic distinctions exist, but the principal ones deal with the differentiation of endo- and ectoplasm, with locomotion and reproduction. In *A. intestinalis* the ectoplasm is scarcely apparent except in the pseudopodia; in *A. vegetabilis* (Figs. 28, 29 and 30, from living specimens) the ectoplasm appears usually to envelope the entire amoeba. Furthermore, *A. intestinalis* is not very active, whereas *A. vegetabilis* is exceedingly active. *A. intestinalis* forms spheroidal spores 0.5 to 1.5μ, whereas *A. vegetabilis* does not form any spores.

EXPERIMENTAL DATA

Since Tyzzer and Fabyan (1920) were able to produce certain lesions of blackhead in young turkeys, chickens, and pigeons by subcutaneous and intramuscular inoculation of tissue from liver lesions of acute cases, it seemed advisable to inoculate these birds with cultures of Heterakis ova, and also with the two species of amoebae isolated and described in this paper. Following the directions of the above named workers explicitly, young turkeys, 5 to 9 days old chickens, and pigeons were inoculated. Small, white, indurated masses or, in some cases, quite extensive reactions were obtained after a few days in the connective tissue under the skin. In no case, however, were any of the lesions of blackhead reproduced. Sectioned material was studied extensively, and it was found that the amoebae associated in the Heterakis cultures and used for these experiments were not modified to resemble *Histomonas meleagridis* Tyzzer (*Amoeba meleagridis* Smith) after gaining admission to the bird tissues. Usually the amoebae were killed off by the tissues or, when found, resembled the amoebae as they appear in culture.

Whereas it has been proven by Graybill and Smith (1920) that one of the factors for the production of blackhead in turkeys and chickens

is present in the Heterakis cultures in the form of the embryonated worms, the results of the experiments here briefly outlined seem to show that the protozoan factor is missing in such cultures.

SUMMARY

1. Commonly one of two species of amoebae or both species of amoebae developed within embryonated Heterakis salt solution cultures. The amoebae were cultivated in pure cultures, and described as two new species, viz., *A. commutabilis* and *A. vegetabilis*.

2. In *A. commutabilis* a modification of the promitotic method of division is described. In this same amoeba interesting nuclear cyclic changes were observed.

3. New cytologic details accompanying and following sporulation were elucidated in *A. commutabilis*. A process of budding was discovered, accompanied and followed by nuclear cyclic changes. A sexual process was not observed.

4. Agamic division, a kind of sporulation, and budding constituted the methods of multiplication in the life history of *A. commutabilis*.

5. Agamic division, probably promitotic, was the only method of multiplication found in *A. vegetabilis*.

6. Turkeys, chickens, and pigeons when inoculated with Heterakis cultures containing one or both species of amoebae, or when inoculated with pure cultures of the two speciaes of amoebae, failed to develop lesions of blackhead.

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EXPLANATION OF PLATE I

Plate I.—*Amoeba commutabilis*. ×813. Figs. 1-5, Living trophozoites. 6, Cyst. 7-11, Fixed and stained preparations showing promitotic division. 12-18, Process of sporulation. 18, Development of spore. (Fig. 12, fixed and stained. Figs. 13-18, stained intravitally.) 19-24, Fixed and stained preparations showing process of budding. 24, Development of bud. 25, Young trophozoite derived from bud. 26-27, Uninterpreted nuclear cyclic changes.

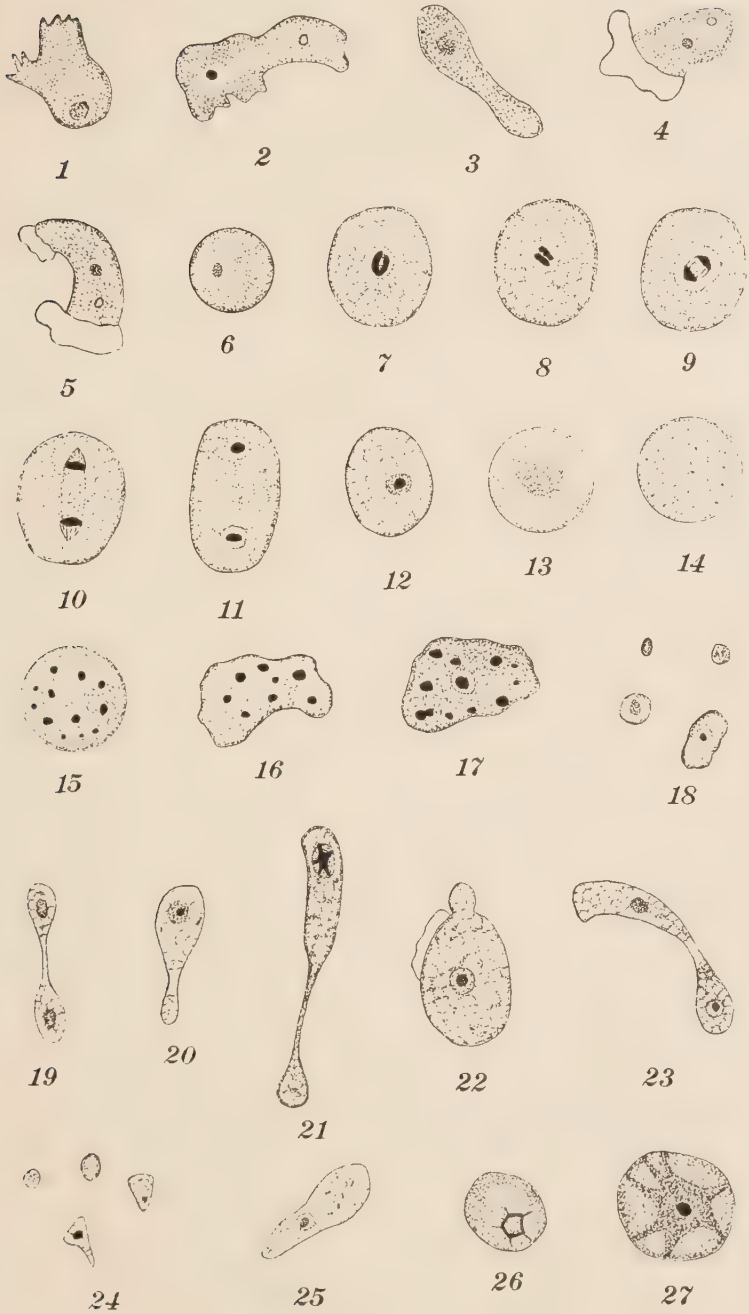


PLATE I

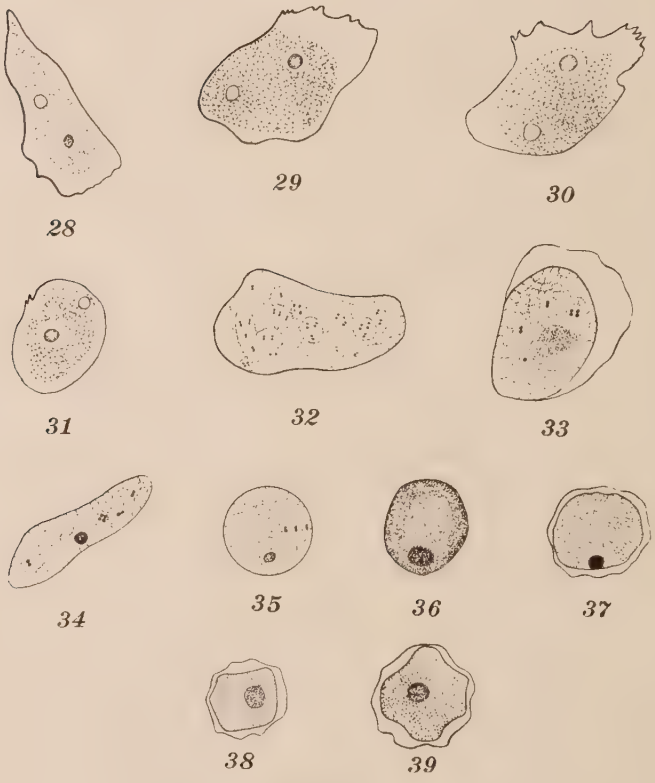


PLATE II

EXPLANATION OF PLATE II

Plate II.—*A. vegetabilis.* ×813. Figs. 28-31, Living trophozoites. 32-34. Fixed and stained trophozoites. 35, Resting stage. Fixed and stained. 36, Early cyst. 37-38. Types of mature cysts.

NOTES ON SOUTH AFRICAN LARVAL TREMATODES *

Ernest Carroll Faust
Peking, China

In previous publications (Faust 1919, 1920, 1920a) I have presented my studies on Cawston's South African larval flukes, have described methods for differentiating these one from the other and have indicated their bearing on life-history problems. Through a grant from the Streatfield Research Fund of the Royal College of Physicians and Surgeons of London, Dr. F. G. Cawston has been enabled to make collections of material which it has been my privilege to study. I desire, therefore, to acknowledge my indebtedness to Dr. Cawston for the opportunity afforded. The present study is published in order to aid in certain life-history studies now being carried on by several investigators in South Africa, as well as to make the data a matter of record.

Cercaria octadena nov. spec. (Fig. 1)

This new form was found in the digestive gland of *Physopsis africana,* collected at Sydenham and at Pinetown. In previous collections from this host at Sydenham, I did not find this furcocercous cercaria. It is one which might readily be confused with the two human schistosome cercariae which I have previously described from this region. It differs, however, in several important points.

In the first place the integument of both body and tail is free from spines. Secondly, the number and disposition of the so-called "mucin glands" differ from those of the two human species. In *C. octadena* there are two pairs of acidophilic glands and two pairs of basophilic glands, the latter being situated anteriorly to the former. These specific differences allow of no uncertainty as to the validity of the species. The worm has a body measurement of 170 by 60μ, while the unforked portion of the tail measures 180 by 40μ and the furci are 85μ long. The parthenita is a sporocyst.

Cawston (1920) has recently raised the question as to whether the cercaria which I described as the larva of *Schistosoma mansoni* might not be in reality that of *S. bovis,* inasmuch as he has not encountered *S. mansoni* clinically and the later species has been described for the locality. In this query Cawston overlooks the fact that the larva which I have described for South Africa as the cercaria of *Schistosoma mansoni* corresponds in all critical points with that also described from

* Contribution from the Department of Pathology, Peking Union Medical College, Peking, China.

Venezuela (Faust 1919: 166), known experimentally to be the larva of *Schistosoma mansoni.* Furthermore, I have actually seen the lateral-spined eggs of *S. mansoni* preserved in the liver gland of *Physopsis africana* which Dr. Cawston collected from Ottawa, Natal, and later sent me. I am certain from my studies, therefore, that both species in question exist in Natal, and that there is a genetic relation between *Cercaria schistosomatis-mansoni* Faust of Natal and the adult *S. mansoni.*

It does seem highly probable, however, that *Cercaria octadena* is the larva of *Schistosoma bovis.* The general appearance as well as the specific characters of the cercaria indicate that it belongs to the group of mammalian schistosomes, while the presence of bovine schistosomiasis in Natal strengthens the probability. Difficulty in actually demonstrating the point lies in the fact that its occurrence thus far has been in mixed infections, along with the larvae of *S. haematobium* and *S. mansoni,* although this difficulty may be obviated by diagnosing both larvae and adults in experimental infection.

Cercaria secobii Cawston 1915 (Fig. 2)

Well-preserved material of *Cercaria secobii* secured from *Physopsis africana* at Pinetown and at Duff's Road, and from Sarnia sp. from the Umbibo River makes it possible to supplement the scant description of this species which I was obliged to give in a previous paper (Faust 1919: 165). Details of the digestive ceca have not been worked out, but there is a minute muscular pharynx behind the well-developed oral sucker. The acetabulum is small. Four pairs of mucin glands in tandem arrangement occupy the middle third of the body. They are neutrophilic in reaction. As is usual for the group their ducts open anteriad, laterad to the buccal cavity. A firm clump of germ-gland cells lies in the region midway between acetabulum and distal portion of the body proper. The entire larva is covered with spines.

Cercaria oblonga nov. spec. (Fig. 3)

This eye-spotted cercaria was secured from the digestive gland of *Tiara tuberculata* collected at Mt. Prospect. The body is oblong-ovate in contour, with a length measurement of 180μ and a width measurement of 65μ. The tail has a plump aspect at its proximal end but tapers out to a conspicuously pointed tip. It is almost twice as long as the body. The body and tail are both without integumentary spines. On the dorsal surface just anterior to the pharynx is a pair of eyespots with optic openings postero-laterad. Likewise, there is considerable pigmentation in the region of the cerebral commissure and over the dorsal and ventral nerve trunks. The oral sucker has a width of 30μ,

with a small buccal cavity. The acetabulum is not well defined. It is probably represented by a glandular complex along the midline somewhat behind the middle of the body.

A long prepharynx leads into a small muscular pharynx. Behind this organ a short esophagus leads into ceca which have been entirely modified into discrete unicellular glands, there being perhaps thirty to forty in each cecal region. While they are morphologically and ontologically different from mucin glands their reaction to dyes leads one to believe they have the same physiological function.

The excretory bladder is a large vesicular structure which opens postero-dorsad through an attenuated duct. Absence of germ glands shows that the larvae is late in maturing. The redia in which the worm develops has a long muscular pharyngeal region but a very small cecum. The birthpore is also inconspicuous.

Cercaria puerilis nov. spec. (Fig. 4)

This minute stylet larva was secured from the digestive gland of *Tiara tuberculata* at Mt. Prospect. It has an ovoid body 80μ long, 53μ wide, and is as thick as wide. The tail is slightly longer than the body. No spines have been found on the integument. The stylet has a delicate piercing tip, a narrow shank and a blunt base. The oral sucker has a diameter of 22μ while the acetabulum measures only one-fourth that amount.

The oral atrium leads directly into a rotund pharynx behind which is found a typical digestive tract. It is lined with a few large epithelial cells. Three large mucin glands on each side of the ceca occupy a good share of the space anterior to the acetabulum. They give an acidophilic reaction and contain prominent granules. A large oval mass just to the left of the acetabulum constitutes the reproductive cell group. The excretory bladder is vesicular. The large cornua arise from it at angles of 120°. The worm develops in a sporocyst. Encystment takes place within a thin chitinous membrane, which is resistant to acids.

Cercaria ingracilis nov. spec. (Fig. 5)

The species is a xiphidiocercaria with a body covered with minute spines and a minute lenticular stylet. The tail is aspinose. The body measures 280 by 170μ and the tail 420μ long. Judging by its shape the movement of the larva is slow and awkward. The specimens were secured from the digestive gland of *Isidora schakoi* collected at Mt. Prospect.

The oral sucker of the worm has a transverse diameter of 54μ, while the acetabulum is slightly smaller. A long prepharyngeal region leads into a relatively large pharynx. Behind this organ a short

esophagus opens into long narrow ceca extending to the subdistal region of the body. The epithelium of the gut is glandular. Outside of the cecal area are masses of granular mucin glands extending all the way from the oral sucker to the midacetabular region. Ducts of these glands empty laterad to the stylet. The excretory bladder is an irregular polygon with small collecting tubules leading from anteriad into the cornua. The excretory pore opens only slightly dorsad to posteriad. The genital cells consist of an irregular-shaped mass dorsal to the acetabulum and extending somewhat anteriad. The parthenita is a sporocyst. Encystment has not been observed.

Cercaria cephaladena nov. spec. (Fig. 6)

Cercaria cephaladena is a stylet larva with very delicately-formed organs. It was obtained from the digestive glands of *Isidora schakoi* at Mt. Prospect. The body is covered with delicate spines, while the tail is aspinose. The stylet is regularly quill-shaped. The body measures 210 by 75μ and the tail 175μ in length. The oral sucker has a transverse diameter of 22μ and the acetabulum 25μ. Leading in from the oral atrium is an elongate pyriform pharynx which opens almost directly into the digestive ceca. These latter are slender tubes extending to the subdistal region of the body. The mucin glands lie in the cephalic area of the worm. They consist of five pairs of basophilic glands with small compact nuclei. The excretory bladder consists of a median vesicular portion which opens posteriad through a slender tube, and an anterior portion lined with glandular epithelium.

The most unique system is the genital complex. It is far advanced for a larva although none of the parts are yet mature. The testes have become separated as distinct spherical masses lying tangentially posterior to the ootype. Prostate and cirrus organs are distinguishable. The vitellaria consist of small masses extending from the pharyngeal region to the line of the posterior testis. Definite vitelline ducts extend transversely to the ootype. These structures taken together clearly distinguish this larva as a plagiorchiine species.

Cercaria humilis nov. spec. (Fig. 7)

This xiphidiocercaria was secured from the digestive gland of *Lymnaea natalensis* at Mt. Prospect and from *L. natalensis* and *Physopsis africana* at Duff's Road. It has a body measurement of 200 by 70μ, and a tail 118μ long. Body and tail are aspinose. The oral sucker has a transverse diameter of 43μ while the smaller ventral sucker is only 29μ in diameter. A long prepharynx leads from the oval cavity to the compact spherical pharynx, which latter organ is 12μ in diameter. Behind this a long slender esophagus bifurcates just in front of the acetabulum to form the ceca. The latter organs

extend to the subdistal margin of the body. There are about thirteen pairs of mucin glands in the body, consisting of nine pairs of granular acidophilic cells lying posteriad and four pairs of *mucoid basophilic cells* situated anteriad. The ducts of these glands are associated in compact bundles. The openings of the ducts near the stylet are small in diameter and close together. The excretory bladder proper is beet-shaped, with cornua arising just laterad from the median line. The pore is dorsoposteriad. The genital cell mass is an irregular structure lying below the sinistral margin of the acetabulum. The parthenita is a sporocyst. Encystment has not been observed.

This larva resembles *Cercaria cawstoni* in certain respects, particularly the type of digestive tract, but differs with respect to kind and number of mucin glands, the stylet, the excretory bladder and the genital complex. These differences in a larval trematode denote a very considerable difference in adult structure, more profound, indeed, than that distinguishing two somewhat related species.

Cercaria vertebraeformis nov. spec. (Fig. 8)

Cercaria vertebraeformis is a large worm exhibiting many points of interest in the morphology of the trematodes. The body is ovate-elongate, while the heavy muscular tail has both a dorsal and a ventral fluted keel. Neither body nor tail possess spines and no stylet has been found. The body measures 270 by 125μ while the tail has a length measurement of 290μ. Oral sucker and acetabulum are equally large, with a longitudinal diameter of 58μ. The transverse diameter is slightly less.

Within the oral atrium is a prepharyngeal region. The pharynx (25μ in diameter) leads into an esophagus impregnated with chitin which has grown in from the orifice and extends inward slightly beyond the forking of the gut. At this point arise true digestive ceca which extend posteriad somewhat beyond the limits of the acetabulum. These ceca are composed of a single layer of epithelium which consists of a syncytial mass of cells. The mucin glands are located laterad to the excretory bladder. A group of about sixteen small polygonal cells with minute nuclei and cytoplasmic strands radiating from the nucleus to the periphery of the cell have their outlets through a conspicuous bundle of ducts that is ovoid with an anterior extension like a truncated pyramid, the lateral angles of which run out into relatively small collecting tubes. The excretory pore is posterodorsad. The genital cell complex consists of an irregular-shaped mass with three arms lying dorsad to the acetabulum. The posterior end of the body has an integumentary thickening giving a mucoid reaction. The sporocyst is sausage-shaped and has numerous constrictions in its outline Encystment has not been observed.

Cercaria macrura nov. spec. (Figs. 9-13)

This unique larva was found in the digestive gland of *Planorbis pfefferi* at Merebank and Mayville. It develops in a large redia with small pharynx, tortuous gut and no "feet." Very few of the features of the body of this worm are sufficiently developed during the cercarial stage to differentiate the fluke *per se* but the caudal appendages are clearly defined.

As the cercaria develops from the germ ball three regions are distinguishable (Fig. 9), an elongate portion which is to develop into the body proper, a cordate portion which is to constitute the main caudal region and a lateral finger-like process which will grow into the caudal cecum. As growth takes place (Fig. 10) the body portion becomes elongate club-shaped. Within the tail a dense ball of glandular cells becomes noticeable, while the caudal cecum, consisting of a column of single cells, becomes hollowed out. In view of the excretory function of the caudal cecum in a related species, Cort and Nichols (1920: 10) have designated it the excretory projection. The opening extends from the distal end of the cecum directly through to the distal margin of the caudal trunk. Later (Fig. 11), the latter region becomes considerably enlarged, the glands become granular with vesicular inclusions and the margins become elytral. At this stage the caudal cecum begins to decrease in size while the body and tail trunk are of maximum size. The body has a length measurement of 180μ and a frontal diameter of 30μ. The oral sucker is 28μ in transection and the acetabulum of equal size. The small pharynx measures 18μ in section. A typically forking digestive tract is visible. Mucin glands and genital complex have not yet developed while the excretory system cannot be made out. The caudal trunk has a gross length of 160μ and a gross width of 100μ. The connection between this region and the outside has now become closed at the distal margin.

The next stage (Fig. 12) shows a distinct tendency toward encystment. The glands have been utilized in the formation of a cyst membrane on the inner surface of the caudal trunk while the body of the worm becomes invaginated into the cyst cavity provided. The elytra become resorbed. The caudal cecum persists. Later (Fig. 13), the worm has become entirely engulfed within its own tail trunk, while the caudal cecum acts as a stipe for attachment to objects near at hand. In this way a larva, ontogenetically young and with only slight differentiation, passes into the encysted stage (agamodistome). Such a condition would not be possible at this stage in a larva depending on the development of cystogenous glands within itself.

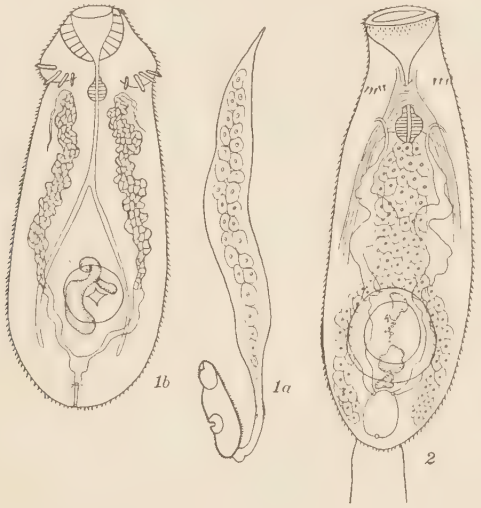
The position of *Cercaria macrura* is difficult to define. It has some features in common with the rhopalocercous larvae, although the fact that the tail is actually used as an organ of protection and encystment

places it in the *cystophorous* group. The mechanism for encystment makes possible the transfer to a second intermediate host which in the case of a related species, *C. cystophora,* is the dragon-fly, *Colopteryx virgo.*

Cercaria caudadena nov. spec. (Text figs. 1a, 1b)

This larva was obtained from the digestive gland of *Planorbis pfefferi* collected at Merebank. From its tail it might be regarded as a megalocercous larva but the body clearly defines it as an echinostome cercaria.

The body of *Cercaria caudadena* is oblong-ovate with a distinct coronal constriction, in which region there is a circlet of not more than twenty-four relatively large spines in a single series. The body



Text figs. 1a, 1b.—*Cercaria caudadena:* 1*a,* lateral view of entire larva, × 50; 1*b,* ventral view of body, × 233.
Text fig. 2.—*Cercaria cucumeriformis,* ventral view of body and proximal portion of tail, × 233.

is entirely covered with minute spines. The tail is aspinose and is several times the length of the body and almost twice the width. The oral sucker has a transverse diameter of 38μ while the acetabulum, situated in the posterior third of the body, is of equal transection.

The dilated oral cavity of the larva becomes constricted as it reaches the region of the pharynx. The latter organ has a transverse diameter of 12μ and lies in the plane of the coronal constriction. A long narrow esophagus runs posteriad, forking half way toward the acetabulum and merging into ceca which extend somewhat beyond the posterior margin of the acetabulum. Granular mucin glands extend from the region of the pharynx to the acetabulum, filling most of the

intermediate space. The excretory bladder is roughly triangular with a narrow tube running caudad and opening through a small pore dorsad. From the anterolateral extensions of the bladder irregular ducts arise, which are enlarged between the acetabulum and pharynx to accommodate the rough crystalloid deposits of the system. The ducts are reflexed in the region near the pharynx in a typical echinostome fashion. The genital complex consists of a mass coiled upon itself in the region of the acetabulum. The tail has a core of large glandular cells of unknown function. The parthenita is a redia with typical pharynx, gut and "feet." Encystment has not been observed.

Cercaria cucumeriformis nov. spec. (Text fig. 2)

Cercaria cucumeriformis is an echinostome larva specifically different from others of this group described from South Africa in that the oral sucker is directed forward instead of ventrad or antero-ventrad. The cercaria was found in the digestive gland of *Isidora schakoi* at Merebank and in *Lymnaea natalensis* taken from the Umgeni River.

The body of the worm is elongate with the anterior end truncate and the posterior end rotund. The body measures 260μ long by 83μ at the greatest transverse diameter. The tail is slightly longer than the body. The collar region is marked by a small number of coronal spines of spiculate type. The oral sucker measures 43μ in transection and the acetabulum, situated far caudad, measures 50μ in diameter.

From the oral cavity the prepharynx leads into a large muscular pharynx behind which is an esophageal region. The ceca arise just anterior to the acetabulum and continue to the subdistal region of the body. Both esophagus and ceca are unique in consisting of glandular epithelium throughout their entire length. A cross section in the region of the esophagus shows from eight to eleven cells surrounding its lumen, but in the ceca the number in section is reduced to three or at most four. The excretory bladder is spheroidal, with pore posterodorsad, and collecting tubes that arise from the anterior wall some little distance from the midline. As they are traced forward they are seen to undulate, with irregular dilatations along the course. They become constricted, however, in the region of the pharynx and turn back on themselves. The genital complex lies dorsal to the acetabulum. There are three large cells, but no definite differentiation has been noted. The parthenita is a redia with no conspicuous characters. Encystment has not been found.

Cercaria catenata Cawston 1917

I have found *C. catenata* in *Isidora schakoi* collected at Merebank.

Cercaria pigmentosa Cawston 1919

C. pigmentosa was found in *Lymnaea natalensis* collected at Sydenham and in *Physopsis africana* at Duff's Road. This larva is undoubtedly the cercaria of a Fasciola, but while both *F. hepatica* and *F. gigantica* occur in South Africa I have been able to demonstrate only the one species of larva.

DISCUSSION

This paper presents ten new species of South African cercariae in addition to clearing up the details of structure in *Cercaria secobii* and giving new distribution records for two other species. There is thus constituted a group of 25 well-defined larval trematodes with the following distribution: amphistome, 1; monostome, 1; echinostome, 7; xiphidiocercariae, 5; furcocercariae, 7; gymnocephalous cercariae, 2; cystophorous cercaria, 1; allocreadine cercaria, 1. I have had an opportunity to study material from all of these species except *Cercaria coma* Gilchrist 1918. In some instances as many as ten or twelve separate collections of one species have been available for use. Since no opportunity was afforded for working on the living material I have had to determine, therefore, (1) what reagent is the most reliable for diagnostic work in this group, (2) which organs are the least distorted by preservation, and likewise, (3) which organs can be depended on in preserved material for specific diagnosis.

With respect to the first query, fixatives containing acid are not successful for long-distance carriage, while five per cent formalin proves eminently satisfactory. Formalin hardens the tissues sufficiently to prevent maceration in carriage and at the same time make the material especially fitted for the study of toto mounts. There is no vagueness of differentiation such as is commonly found in the formalin preservation of large mammalian organs.

A comparison between the study of living and preserved larval trematodes consists primarily in the fact that the living flukes permit a study of movement and of the flame-cells, which the preserved material does not, whereas the preserved material frequently gives a clearer picture of the germ-cell complex. Usually the excretory bladder and the primary collecting tubules are likewise more conspicuous in the living specimens, while the digestive system, including mucin glands, is more clearly depicted in stained mounts. If it were possible, therefore, to study an abundance of living material the excretory system offers a definite basis for diagnosis and differentiation. But this opportunity is frequently not afforded, so that it is even more important that a conservative system be found which permits diagnosis in preserved specimens. The gross external anatomy is obviously not to be considered, and the digestive tract *sensu stricto* is often not specific. The genital complex is not always dependable, due to the fact

that it frequently develops late in the ontogeny of the worm. On the other hand, a study of South African as well as many other species leads me to believe that the mucin glands offer a system highly differentiated within a particular group, yet constant within the species. It is almost entirely on this basis that a differential diagnosis has been made among the three species of human schistosome cercariae and it is this system that offers in this study the differentiation between these three schistosome species and *Cercaria octadena,* the probable larva of *Schistosoma bovis.*

The fact that certain of these glands constantly have a basophilic reaction (and can be readily differentiated by Best's carmine stain as well as hematoxylin stains) and that others are acidophilic in reaction, not only makes the differentiation easier but presents a most important problem in connection with the physiology of the larva and the pathology of the host. In this connection it may be mentioned that the differentiation of these two varieties of digestive glands has made the term mucin gland untenable when applied to these glands as a whole. I wish to suggest, therefore, that the term *cephalic gland* (Cort 1919: 501) be used in place of mucin gland, so that one may refer to basophilic, neutrophilic or acidophilic glands of this type without confusion or misinterpretation. A comparative study of the effects of these glands on host tissues indicates that basophilic and acidophilic glands are complementary in function and that a larva possessing the two differentiated types can penetrate the host much more effectively than one possessing only one type. The schistosome cercariae are notable examples of the dimorphic type of staining reaction.

Leiper (1915: 188) has found some twenty-five species of cercariae in his study of the schistosome problem in Egypt. While the larvae of *Schistosoma haematobium* and of *S. mansoni* occur in both Egypt and Natal, the other described cercariae are specifically different. It seems quite probable that several of the species reported for Egypt are present in South Africa but have not yet been collected, as for example the larval amphistomes *Cercaria pigmentata* and *C. aegyptiaci,* the adult forms of which have been reported for Natal. It is less likely that some of the larval flukes first described from South Africa will be found in Egypt, since the latter country has been extensively surveyed for trematodes. A collection of material from tropical West Africa would undoubtedly yield important data in connection with the two areas now relatively well surveyed.

SUMMARY

1. Further collections of larval trematodes from South Africa have made possible the description of ten new species.

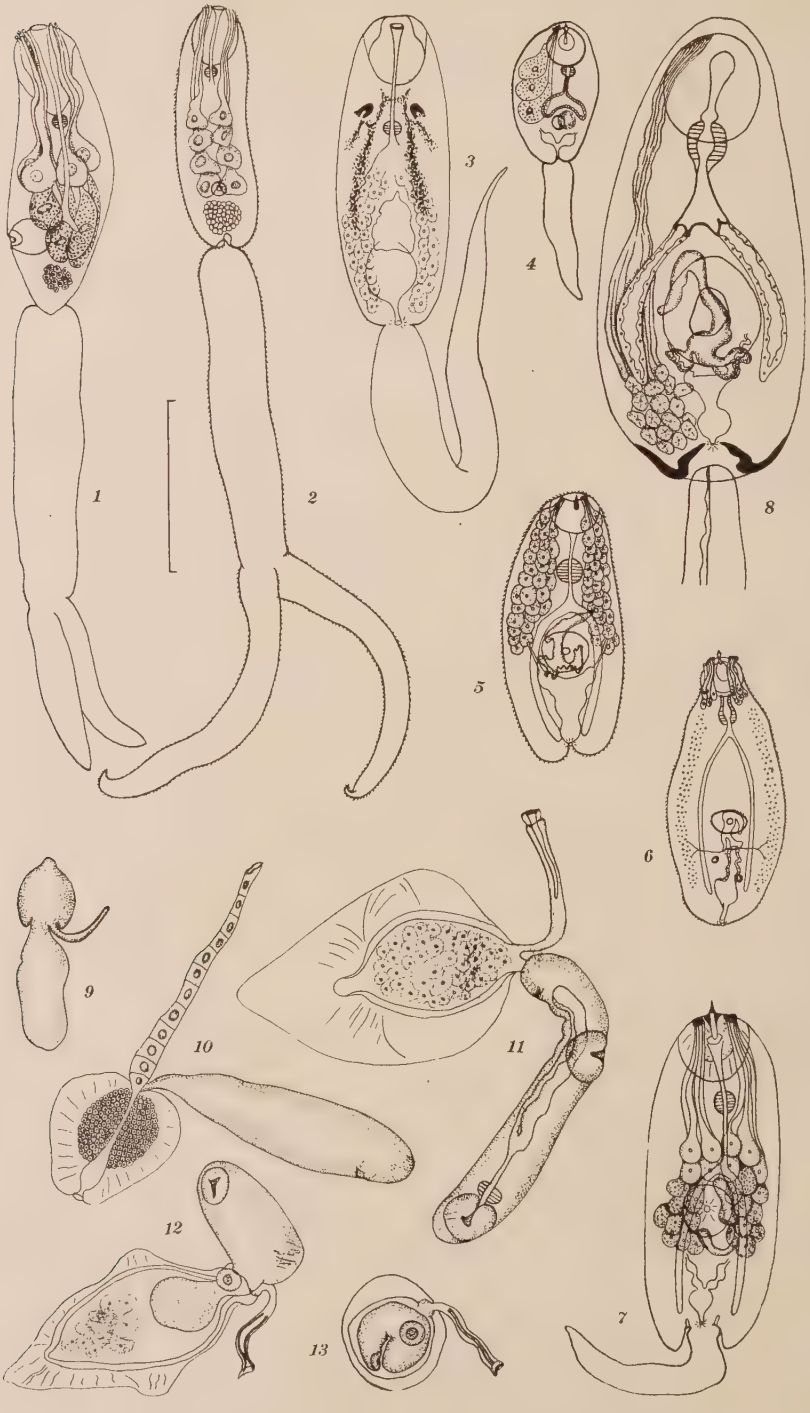


PLATE III

2. *Cercaria octadena,* the probable larva of *Schistosoma bovis,* is readily differentiated from the larval stages of the two human schistosomes in South Africa on the basis of its digestive glands.

3. The digestive glands are the most important structures for differentiating preserved material.

4. Since the term mucin gland is applicable only to the digestive gland giving a basophilic reaction the term *cephalic gland* (Cort) is suggested to designate all such glands, including those giving basophilic, neutrophilic and acidophilic reactions.

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Explanation of Plate III

Fig. 1.—Lateral view of *Cercaria octadena.*
Fig. 2.—Ventral view of *C. secobii.*
Fig. 3.—Ventral view of *C. oblonga.*
Fig. 4.—Ventral view of *C. puerilis.*
Fig. 5.—Ventral view of *C. ingracilis.*
Fig. 6.—Ventral view of *C. cephaladena.*
Fig. 7.—Ventral view of *C. humilis.*
Fig. 8.—Ventral view of *C. vertabraeformis.*
Figs. 9-13.—Progressive stages in the development of *C. macrura.*

Note.—The line has a value of 0.1 mm. in all figures except fig. 5, where it has a value of 0.18 mm.

RHYNCHOBOTHRIUM INGENS SPEC. NOV. A PARASITE OF THE DUSKY SHARK (*CARCHARHINUS OBSCURUS*) *

EDWIN LINTON

On August 14, 1905, I examined a lot of cestodes from a dusky shark which had been taken a few days before at Menemsha Bight. The shark measured 10 ft. 10 in. in length. The gills of a sword fish were found in the stomach. This fact, of course, is not to be interpreted as proof that the shark had eaten a sword fish. The gills had doubtless been picked up by the shark from offal thrown overboard by fishermen.

The cestodes found in the spiral valve were:—numerous specimens of *Crossobothrium angustum,* a few examples of *Phoreiobothrium lasium,* both being species of frequent occurrence in the dusky shark, and seven large strobiles of a species which, on account of the character of the hooks, suggested *Rhynchobothrium speciosum,* a species originally described from scoleces obtained from cysts in various teleosts.

These strobiles were much crumpled, since the spiral valve from which they were obtained had been lying in a weak solution of formaldehyde for a few days; tho contracted they were 410, 470, 500, 570, 600, 640, and 715 mm. long.

At the time of collecting the following data were noted:

Bothria somewhat broadly heart-shape, emarginate on posterior free border, widely flaring; breadth of head (marginal view of spreading bothria) 2.5 mm., of neck 1.8 mm.: length of neck 11 mm.; length of bothrium 1.8 mm., breadth 2.2 mm.; distance to first segment 80 mm.; maximum breadth of strobile 9 mm.; posterior segment, length 9 mm., breadth 7 mm. Hooks appear to agree with *R. speciosum.*

No report was made of this find further than the record of *Carcharhinus obscurus* as the final host of *R. speciosum* (U. S. Fisheries Bulletin, vol. xxxi, Part ii, p. 588).

On account of the massive character of the strobile but little knowledge of the structure could be gained from a study of unsectioned material.

The best results were obtained from sections made from pieces stained in toto in borax carmine, and further stained on the slide in a weak solution of indigo-carmine in 95% alcohol. In sections treated with indigo-carmine the following structures, which were either unstained, or but lightly stained by the carmine, were stained blue:—all muscle fibers, cuticula, fibers in the subcuticula, spermatozoa in the

* Contribution from the United States Biological Station, Woods Hole, Mass., and the Zoological Laboratory of the University of Missouri.

vas deferens, seminal vesicle, seminal receptacle, as well as those which surrounded the maturing testes. In like manner sharp differentiation was obtained between the cells of the shell gland and of the ovary. The cells of the ovary were deeply stained by the carmine and showed no trace of blue, while the nuclei only of the cells of the shell gland were red, the cytoplasm being strongly stained by the indigo. In cross sections of the spermduct and the oviduct the walls were blue and the surrounding gland-cells were red.

Scolex.—The bothria are two in number, broader than long, somewhat broadly heart-shape in general outline with the smooth margin slightly raised, and the posterior free border usually flaring. The bothria correspond to the flat surfaces of the strobile, i. e., dorso-ventral. In preserved specimens the neck has a tendency to twist so as to make the bothria appear to correspond in position to the narrow margins of the strobile. The neck is elongated, subcylindrical, of nearly uniform dimensions from a point near the bothria to the anterior ends of the slender contractile bulbs, where it increases slightly in breadth to remain unchanged to the base of the bulbs. In a specimen mounted in balsam the scolex measures 10.4 mm. from the anterior end of the bothria to the posterior end of the bulbs; a proboscis was estimated to be 2.8 mm. in length; bulbs, not very clearly outlined, measured 3 mm. in length and 0.4 mm. in diameter. Bulbs dissected out of another specimen measured 3.5 mm. in length and 0.36 mm. in diameter. A bothrium removed from the scolex and flattened measured 1.68 mm. in length and 1.89 mm. in breadth.

The proboscides are but partly everted, and moreover are much contorted, so that it is difficult to make a satisfactory interpretation of the plan of arrangement of the hooks. So far as I have been able to make it out the plan is as follows (Fig. 5): On the lateral aspect of the proboscis there is a longitudinal row of small, slender spines placed by twos in tandem (a). This row is flanked on one side by a row (b) of spines which vary greatly in shape. At the point represented in the figure, which is about one millimeter from the base of the proboscis, they appear in front view as short, broad, triangular spines corresponding in number to row (a) but uniformly placed, close together, the base of one slightly overlapping the base of the spine in front of it. Towards the base of the proboscis the spines of this row develop wide wing-like basal supports, from which the spine proper rises as a slender process which at first bends forward then turns towards the base of the proboscis in a short recurved hook (Fig. 9). On the other side of row *a* there is a row of slender hooks (*c*) smaller than *a*, and one half as many, each hook in this row corresponding in spiral arrangement with the anterior hook in each tandem pair in row *a*. Next to *c* is a series (*e*) of longitudinal rows, appearing in the spiral as five strongly

recurved, stout hooks, increasing in size in the direction away from *c*. The remainder of the spiral (*d*) is another series of longitudinal rows appearing in the spiral as five long, stoutish hooks, growing larger, in a direction away from *b*. The larger hooks in series *d* come to resemble those in series *e*. A spiral of one circumference, according to this interpretation, consists of the anterior hook of a tandem pair of *a*, one from row *b*, five in series *d*, five in series *e*, and one in series *c*. The posterior hook in each tandem pair of *a* and each alternate hook in *b* lie between adjacent spirals. The length of the longest hook is 0.075 mm.

Cross sections of the scolex in the region of the contractile bulbs show that these muscular organs have very thick walls on the inner side, which, on account of the criss-cross arrangement of the diagonal muscle fibers, looks something like sections of cardiac muscle. The wall is thickest on the side of the bulb which is next the axial center of the scolex, and thins out in each direction, until, at the lateral border it is made up of only the external sheath and the lining of the bulb cavity. Measurements made on one bulb showed the thickness of the inner wall to be 0.15 mm. and the outer wall at its thinnest point, 0.006 mm. The retractor muscle which inverts the proboscis is attached near the anterior end of the contractile bulb.

The neck of the scolex at its anterior end, that is, just behind the bothria, is elliptical in section with the longer axis dorso-ventral. The two diameters in a section made at this point measured 1.20 and 0.68 mm. respectively.

In a series of sections of the scolex clusters of what looked like oval cells with indistinct nuclei were noted in the central portions of the sections. The larger ones measured 0.036 by 0.015 mm. and were deeply stained. They represent in the scolex an axial core which began about 0.55 mm. from the anterior end and continued for a little more than a millimeter, with occasional scattering representatives in succeeding sections. On account of the fragmented condition of many they were interpreted to be calcareous bodies, altho no trace of concentric structure could be seen in any of them.

About at the point where the axial group disappears true ganglion cells make their appearance in two lateral groups. These ganglion cells are approximately circular in outline, their cytoplasm is finely granular and lightly stained. The majority of them are from 21 to 24μ in diameter. The number in a lateral group in the earlier sections of the series is about 50, increasing in succeeding sections. The maximum was about 80. These lateral groups continue to be represented by many cells for a distance equivalent to about 2 mm. in the neck of the scolex, when their number is abruptly reduced. Groups of a half a

dozen or less appear in the sections back to the first appearance of the contractile bulbs where their number increases to 12 or more. They continue to the base of the contractile bulbs (Figs. 1 and 4).

The excretory vessels form a net-work of anastomosing vessels in the bothria. At the base of the bothria they merge into longitudinal vessels of which 12 or more were noted in a section made about midway between the bothria and the contractile bulbs. Behind the contractile bulbs the longitudinal vessels of the scolex unite to form the four lateral vessels, characteristic features of the cestode strobile. These are: one larger, ventral, and one smaller, dorsal, vessel near each margin. Of these the larger is the more laterally placed and lies between the smaller vessel and the lateral nerve cord. Both vessels pursue a somewhat tortuous course, the smaller especially so (Figs. 2, 4, 12, Text fig. A).

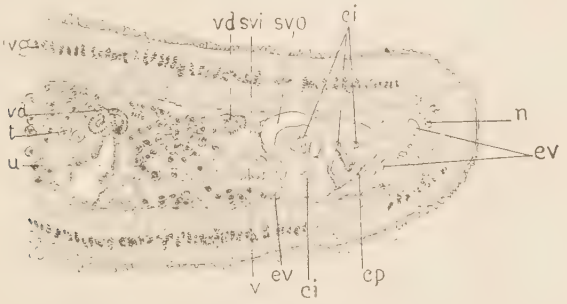

Fig. A.—Transverse section of young proglottis showing cirrus pouch, seminal vesicles, vagina, uterus, etc.; dorso-ventral diameter 1.26 mm.

However, there are some variations from the ordinary arrangement. In the series from which Text fig. A was made, transverse sections of a young proglottis, the largest excretory vessel is dorsal and passes to the dorsal side of the cirrus pouch, dividing into two or more branches in passing, the branches uniting after passing the cirrus pouch. There were three or more small vessels which were ventral. In a transverse series of an adult proglottis only the larger vessel was clearly indicated. It passed without dividing on the dorsal side of the cirrus pouch. In a few sections a smaller vessel was seen lying very close to the larger vessel on its dorsal side. In a series of sagittal sections of immature proglottides the larger vessel is distinctly ventral, and the smaller, dorsal.

In a series of adult proglottides the larger vessel is not distinctly either dorsal or ventral. It passes on the dorsal side of the cirrus pouch. The smaller vessel is dorsal.

Strobila.—The general aspect of the strobile suggests that of the Bothriocephalidæ, the proglottides being indistinctly indicated, even

those which are fully mature and have the uterus filled with eggs and occupying the greater part of the interior of the proglottis. The habit is rather thickish thruout. In preserved specimens the margins, in the median region especially, are finely wrinkled, but the middle of the flat faces of the strobile, dorsal and ventral, is for the most part smooth, with a tendency to develop longitudinal wrinkles in the anterior regions (Fig. 15).

In a specimen 680 mm. in length, the diameter just back of the contractile bulbs, at the beginning of the strobile proper, is 2.25 mm. From this point the strobile increases gradually and rather uniformly in width for some 300 mm., when the greatest width, 8 mm., is attained. From that point to the posterior end the breadth remains about the same; a few of the posterior segments which are filled with eggs, are thicker and not quite so broad. The posterior segment is 7 mm. long and 7 mm. broad, and nearly 3 mm. thick. The first segments with ova, so far as their presence could be made out, were 380 mm. from the anterior end. Here they could be seen as a dark central mass thru the thick, but partly translucent, wall. The proglottides are but faintly outlined at best, but by careful study with the aid of the hand lens they could be made out. The first recognized were 100 mm. from the anterior end, and were 3 mm. broad and 0.75 mm. in length.

The genital openings are asymmetrically marginal and irregularly alternate. They are rather difficult to make out since, unless the cirrus is everted, each is at the bottom of a deep notch which is often by contraction of the proglottis reduced to a thin line indistinguishable from the other marginal wrinkles of the proglottis. In young and maturing proglottides the genital aperture is situated a little behind the middle of the length; in older segments it is about, or a little in front of, the posterior third of the length. The lateral margins of the posterior proglottides are nearly entire. Toward the posterior end the posterior margins of the proglottides can be seen to possess a free but very narrow border. The ventral uterine openings, which were demonstrated in sections to lie near the middle of the anterior border of the proglottis, were not seen in any case in the macroscopic study of the strobile (Figs. 13, 12).

When the strobile is allowed to become slightly dry it shows under the hand lens the surface covered with what appear like minute scales. In sections these are seen to be cuticular papillae, which are spine-like and point posteriad; they evidently function to help hold the strobile in place and hence tend to relieve the drag on the proboscides when they are acting as an anchorage. This structure is in harmony with the long and heavy strobile which follows the thick walled structure, and the tendency of the ripe proglottides to remain attached. No free proglottides were found, and the ripe proglottides showed no tendency to separate from the strobiles.

The cirrus was everted in but few of the proglottides. It is smooth, tapering, and rises from the larger end of a funnel-shaped base. The shape of this basal portion suggests that it may function as an adhesive sucker-like organ in copulation. In a segment measuring about 5 mm. in breadth, and 3.75 mm. in length, the middle of the base of the cirrus was 1.3 mm. from the posterior end; the length of cirrus, including basal portion 2.32 mm., the length of basal funnel 0.45 mm., the diameter of funnel at base 0.21 mm., at the outer extremity, which forms a collar from which the cirrus proper rises, 0.60 mm., the diameter of the cirrus proper at base 0.30 mm., at tip 0.16 mm. (Fig. 14).

The cuticular papillary spines, as shown in a mounted specimen under moderate magnification, are short, conical, bluntly pointed, translucent, horn-like in structure, and strongly deflected posteriorly. They are somewhat variable, but the better developed ones measure 0.07 to 0.09 mm. in length, and 0.03 mm. in diameter at base, tapering to 0.015 mm. at tip.

Anatomy of Proglottis.—The wall of a mature proglottis is relatively thick. In a section measuring 1.15 mm. in the shorter diameter, it was 0.3 mm. thick exclusive of the cuticular papillæ, which were rubbed off from portions of the section. They would add about 0.07 mm; that is, 60% of the thickness of the segment is represented by the walls. The thickness of the layers is slightly variable. The lobes of the vitellaria, for example, present an irregular outline, especially where they join the sub-cuticular layer, into which they penetrate to greater or less degree. Measurements made in a transverse section of a segment with eggs gave the following result: Thickness of cuticular layer, exclusive of papillæ, 0.006 mm., of sub-cuticular layer 0.054 mm., of vitelline layer 0.090 mm., of longitudinal muscle layer 0.108 mm., of circular muscle layer 0.012 mm.

The wall is relatively thicker in immature than it is in mature proglottides; in a proglottis 1.12 mm. thick without vitellaria but with testes and rudiments of other genitalia the wall was 0.42 mm. thick, distributed as follows: cuticula, exclusive of papillae, 0.006, subcuticula 0.13, longitudinal layer 0.27, circular layer 0.014 mm.

The general anatomy of the reproductive organs is shown in sketches of sections and in diagrammatic restorations.

The cirrus has already been described. When retracted it is seen to enter the common duct from the anterior side. The cirrus-pouch is large and in the retracted state of the cirrus extends rather more than half way from the lateral margin to the median line of the proglottis. The vas deferens is a much convoluted tube between the cirrus-pouch and the middle of the segment. It passes to the dorsal side and lies in convoluted folds on the dorsal side of the uterus along the median axis from a point a little back of the middle of the length of a mature

proglottis to the ovary. The testes are very numerous and fill all the space bounded by the circular muscle layer not occupied by the other genitalia. They are not completely interrupted at the junction of proglottides with each other. This is best seen in sagittal sections. The testes are the first of the reproductive organs to appear and in the younger segments fill practically all the space in transverse sections inside the muscular walls. The male reproductive organs mature before the female. In mature segments the testes are conspicuous organs, appearing as globular cell masses, deeply staining in carmine, and surrounded by spermatozoa, which present the appearance of concentric layers of fine fibers.

The vagina opens on the margin of the proglottis at the common genital pore, which, when the cirrus is retracted, is at the bottom of a deep notch. It lies along the posterior border of the cirrus pouch, and is a relatively large tube. In a sagittal section made at the level of the base of the cirrus bulb the section of the vagina occupied one-fifth of the thickness of the segment, and was filled with spermatozoa. The vagina follows a course nearly at right angles to the lateral margin as far as the middle line where, as the relatively large seminal receptacle, it lies on the ventral side of the segment on the median line as far back as the anterior border of the ovary. A slender tube, the sperm duct, with rather thick walls extends from the base of the spacious seminal receptacle to the germ duct. It rises from an inwardly projecting papilla on the wall of the receptacle. After making a sharp turn, which forms nearly one coil of a spiral, it proceeds back in a curved course to the germ duct which it joins at the posterior end of an enlarged portion of the duct near the ovary. A very short distance back of the point where the germ duct is joined by the sperm duct a vitelline duct enters. The vitelline ducts from the two sides appear to join in a common duct shortly before entering the oviduct. The germ duct for a short distance back of the point where it leaves the ovary (germarium), which is on the ventral side, is enlarged, with thick walls surrounded by conspicuous gland cells. It is thick-walled until a short distance back of the point where it is joined by the vitelline duct. It then passes to the dorsal side where it is disposed in several coils. Here its walls are thin. These coils of the oviduct lie in a space which is filled with parenchyma characterized by very few small cells in a mesh of exceedingly fine fibers. This space is surrounded by the cells of the shell gland, which are large, oval, or fusiform with conspicuous nuclei. The diameter of the germ cells is 6μ, while the cells of the shell gland are 18μ long. The earlier coils of the thin-walled portion of the oviduct contained only scattered yolk cells. The duct became more and more crowded with yolk cells, and considerably enlarged, ova and masses of yolk cells lying together in its lumen. The anterior coils contained only ova and merged into the uterus proper.

The uterus in immature proglottides is represented by a slender tube which lies on the ventral side along the median line. It communicates with the exterior by a ventral pore near the anterior end of the proglottis. In young adult proglottides in which ova have made their appearance the uterus is more or less spacious, with a tendency to become sacculated. As the proglottides mature these sacculations extend laterad until, in the posterior segments, all the interior space within the muscular layers is occupied by the uterus crowded with eggs. The eggs are elliptical-ovate, and thin shelled with little or no variation in the size, measuring 60μ by 30μ.

The conspicuous vitelline glands lie between the layer of longitudinal muscles and the subcuticula. They form a relatively thick layer which is interrupted only over a small area dorsal and ventral to the shell gland and ovary in mature joints. The vitellaria are practically continuous at the margins, and at the anterior and posterior ends of the joints, where sagittal sections show at some places the vitelline glands of adjacent joints merging imperceptibly into each other, at others separated by very thin septa. The ovary has two lobes united at the median line. The whole organ occupies approximately one-fifth of the length and a little more than half the breadth of a mature proglottis, and lies at the extreme posterior end.

THE CLASSIFICATION OF THE TETRARHYNCHIDAE

The classification of those cestodes which are characterized by the presence of four eversible proboscides armed with hooks is still in a somewhat unsettled condition.

The genera which I have recognized in previous papers are: Rhynchobothrium de Blav., Otobothrium Lt., Tetrarhynchus Rud., and Synbothrium Dies.

These genera may be recognized from the scoleces alone by the following characters:

Bothria 2
- Bothria plain ..... Rhynchobothrium
- Bothria with two eversible pit-like organs at posterior border .. Octobothrium

Bothria 4
- Bothria lateral (dorso-ventral) ..... Tetrarhynchus
- Bothria terminal ..... Synbothrium

The generic name Rhynchobothrium is not used by some recent writers.

Following is the classification of the Tetrarhynchidæ proposed by Pintner (1913):

1. Eutetrarhynchus Pint. 1913
   Type: *Eutetrarhynchus ruficollis* (Eysenh.)
   Spiral valve of *Mustelus laevis*

2. Stenobothrium (Diesing 1863)
   Type: *Stenobothrium linguale* (Cuv.)
   Stomach of *Mustelus*
   Tetrarhynchus tenue, T. robustus, and *T. bisulcatum* of my papers fall in this group
3. Lakistorhynchus Pint. 1913
   Type: *Lakistorhynchus benedeni* (Créty)
   (= *tenuis* Ben. = *gracilis* Dies.)
   Spiral valve of *Mustelus*
4. Halysiorhynchus Pint. 1913
   Type: *Tetrarhynchus shipleyanus* Pint. 1913
   (New name for *T. ruficollis* Sh. and Horn. 1906)
   From *Trygon walga*
5. Sphyriocephalus Pint. 1913
   Type: *Sphyriocephalus viridis* (Gu. R. Wagner)
   Stomach of *Centrophorus granulosus* and *Scymnorphynus lichia*
6. This generic group is not given a name by Pintner, who simply designates it as the "*Attenuatus*" Group, to which belong: *Tetrarhynchus attenuatus, grossus, megacephalus, Coenomorphus,* and other forms.
7. This group, also without a formal generic title, is made to accommodate *Otobothrium* Lt.

While recognizing the need of a revision of my contributions to the literature of this group, I do not propose to venture upon the task at present. On account of the wide distribution of these cestodes in the encysted stage, where scoleces may be found fully developed, but with no proglottides, it is highly desirable that whatever classification is devised, it should be usable with the encysted stages for the larger groups, preferably genera. It is true that there is great diversity of form and habit in the strobiles, but it is not likely that scoleces which agree in details of structure, including the armature of the proboscides, will be found to differ generically in their strobiles. It is of great importance that descriptions of scoleces should, as far as the material will enable it to be done, include detailed accounts and figures of the hooks, with measurements, also measurements of the different parts of the scolex.

The species under consideration possesses some characters which suggest a form which I have recorded, from scoleces obtained from cysts, as *R. speciosum.* Upon comparing the scolex of this species with scoleces of *R. speciosum,* however, it is seen that we are dealing with a different species. On the other hand a comparison with unidentified species of the genus *Rhynchobothrium* it was noted that a scolex obtained from a cyst in *Mola mola* (Linton 1901) agrees very closely with this species. Unfortunately I am unable to find the specimen in my collection upon which the brief description was based and from which the figures were made. There is a second longitudinal row of slender hooks placed by twos in tandem, represented in the figures

which I do not find in my study of the hooks of the specimens described in this paper. It should be added that this second row of hooks placed by twos is characteristic of *R. speciosum.*

The proboscides of *R. speciosum* are longer and more slender than the proboscides of *R. ingens.* Thus the proboscis of *R. ingens* was estimated to be 2.8 mm. in lengeth, and at one point measured 0.2 mm. in diameter, including hooks. The proboscis of a scolex from a cyst found in *Alutera schoepfii,* which agrees with *R. speciosum,* measures about 2 mm. in length and 0.1 mm. in diameter, including hooks. The length of the proboscis in scoleces identified as *R. speciosum* has been estimated in some cases to be as much as 4 mm. The length of the longest hooks in *R. ingens* is about 75μ, of *R. speciosum,* about 50μ.

Rhynchobothrium ingens sp. nov.

Bothria two, neck of scolex elongate, subcylindrical, merging into strobile without collar (acraspedote, Pintner); characters of genus.

Bothria broader than long, emarginate on posterior border, flaring in marginal view. Proboscides rather long, armed with hooks of considerable variety of shape, arranged in about 13 longitudinal rows, of which 5 are of stout, recurved hooks placed near together, 5 of longer, straight hooks slightly curved at the extremity, also placed near together, one row of small, slender-pointed curved hooks adjacent to the group of stout, recurved hooks, one of very variable short hooks with broad bases, adjacent to the group of long straight hooks; between the two latter rows is a row of small slender-pointed hooks placed by twos in tandem. This description for the hooks near the base of the proboscis. Contractile bulbs slender slightly arcuate.

Strobile long and thick, 700 mm. or more in length, and 7 mm. or more in breadth; proglottides rather indistinct except toward the posterior end; reproductive apertures irregularly alternate, behind middle of length of progloctis: cirrus smooth, with a funnel-shape collar at base. Vagina posterior to cirrus. Uterus with a minute opening to the exterior situated ventrally near the anterior end of the proglottis on the median line. Ripe proglottides not separating easily, if at all, from the strobila. Cuticula of strobila raised into minute papillary spines.

bl contractile bulbs.
cb calcareous body.
ci cirrus.
cp cirrus pouch.
ev excretory vessel.
evd dorsal excretory vessel.
evv ventral excretory vessel.
ga genital aperture.
gc ganglion cell.
gd germ duct.
n nerve.
o ovary (germarium).
ov oviduct.
pr proboscis.
prs proboscis sheath.
sd sperm duct.
sg shell gland
sr seminal receptacle.
svi inner seminal vesicle.
svo outer seminal vesicle.
t testis.
u uterus.
ua uterine aperture.
v vagina.
vd vas deferens.
vg vitellaria
vd vitelline duct

I am indebted to Mr. George T. Kline, Biological Artist of the University of Missouri, for the greater part of the work involved in the preparation of illustrations for this paper.

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Explanation of Plate IV

Plate IV.—Fig. 1.—Scolex of *Rhynchobothrium ingens.* Diagrammatic sketch of scolex, marginal view, showing distribution of ganglion cells; length of scolex 9.5 mm.

Fig. 2.—Transverse section of bothria, showing anastomosing excretory vessels, retracted proboscides, etc. Dorso-ventral diameter 1.16 mm.

Fig. 3.—Enlarged view of section of border of Bothrium.

Fig. 4.—Transverse section of neck of scolex showing ganglion cells, excretory vessels, etc. Shorter diameter of section 1 mm.

Fig. 5.—Diagram of proboscis, split longitudinally and partly flattened; for significance of letters a-e, see text.

Fig. 6.—Bothrium, front view; breadth 2 mm.

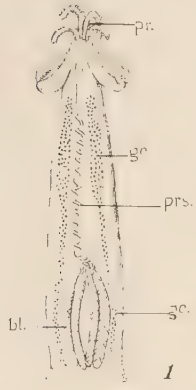
Fig. 7.—Two of larger hooks of series b, Fig. 5, seen in side view, optical section.

Fig. 8.—Hook from series e, Figure 5.

Fig. 9.—Hooks from series b, Figure 5.

Fig. 10.—Hook from series d, Figure 5.

Fig. 11.—Median region of transverse section made near the posterior end of a mature proglottis, dorso-ventral diameter 1 mm.



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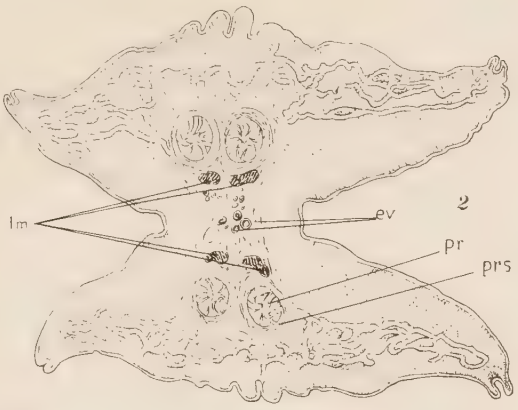

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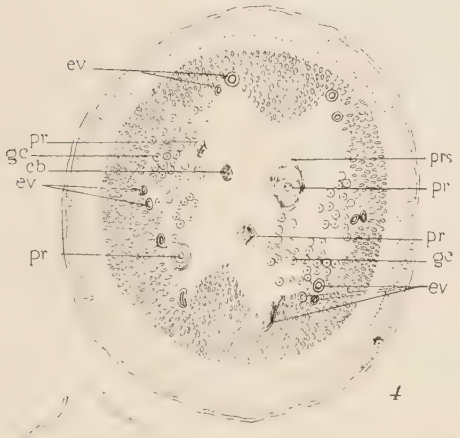

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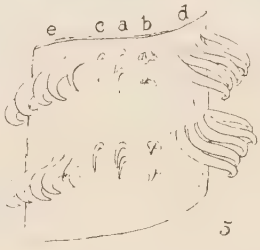

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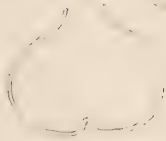

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PLATE IV

Explanation of Plate V

Plate V.—Fig. 12.—Stereogram of mature proglottis, ventral view.

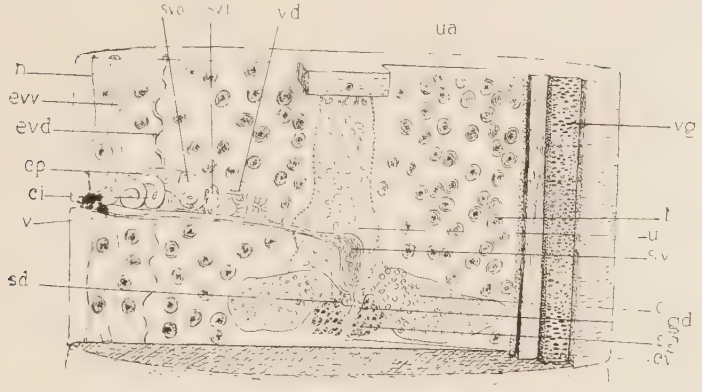
Fig. 13.—Median sagittal section at junction of two mature proglottides: dorso-ventral diameter 1 mm. Three adjacent sections were used in order to show the sperm duct and vitelline duct joining the germ duct.

Fig. 14.—Enlarged view of everted cirrus, length 2.3 m.m.

Fig. 15.—Portion of strobile representing four proglottides. Note that the genital aperture is visible in the lower, while it is not visible in the upper proglottis. This is because the apertures are not quite symmetrically placed on the margins of the proglottides; breadth 5.5 mm.

Fig. 16.—Schematic sagittal view of reproductive organs of adult proglottis in median region of proglottis.

For explanation of lettering on all figures see p. 31.



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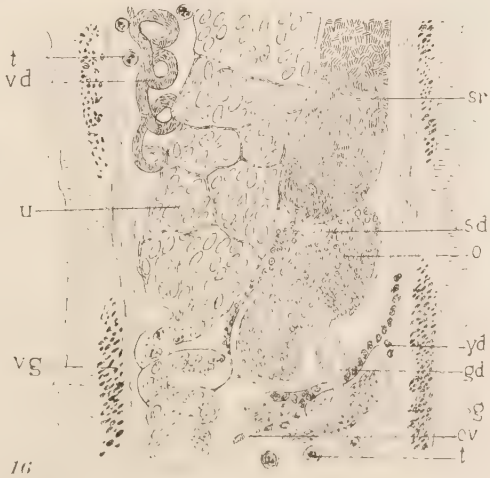

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PLATE V

NOTES ON TWO GENERA OF ECTOPARASITIC TREMATODES FROM FRESH-WATER FISHES*

H. J. Van Cleave

Because of their economic importance the trematodes infesting the general body surface and gills of fishes have received considerable attention at the hands of European investigators. In spite of their importance, there have been extremely few records of their occurrence on fresh-water fishes of North America. Many of the ectoparasitic trematodes are so minute and in life are so translucent that they entirely escape detection by the casual observer or the general collector. With few exceptions the records of ectoparasitic trematodes from fresh-water fishes of North America assume the specific identity of the North American and European forms, as indicated in the following summary:

A trematode epidemic among young fishes under cultural conditions and a general infestation of the wild native fishes were reported from the Craig Brook Station, Maine, in the report of the U. S. Commissioner of Fish and Fisheries for 1899. In this instance no clue is given to the species or even to the genus of the parasites. Later (1901), C. G. Atkins in writing of the same epidemic attributed the parasites to the species *Gyrodactylus elegans*.

Pratt (1919:7) mentions an infection "discovered on the gills of the rainbow trout in the New York State hatchery at Cold Spring Harbor, Long Island. Practically all the trout a year old or more were infected, in many instances in such large numbers that the gills were shriveled and functionless." In this connection Pratt has given a generalized figure of a representative of the genus Gyrodactylus but no specific determination of the parasite.

As a result of his studies on Canadian fresh-water fishes, Cooper (1915:190) reported the occurrence of four species of ectoparasitic trematodes from this continent. In his collections three genera: Gyrodactylus, Ancyrocephalus, and Diplobothrium, were represented. But a single specimen of Gyrodactylus was encountered in the course of his work. This individual was ascribed to the species *G. medius* Kathariner, but in his discussion Cooper brings out data which seem to preclude the possibility of his specimen belonging to this species. He mentions that "The large hooks in the posterior disc, however, are relatively simpler and larger than in Kathariner's *G. medius* and *G. gracilis* being

* Contributions from the U. S. Bureau of Fisheries Biological Station, Fairport, Iowa, and from the Zoological Laboratory of the University of Illinois, No. 186.

54μ in length. They and the sixteen smaller peripheral hooks, each 34μ in length, compare more favorably, as to size, with Wegener's description of *G. elegans* v. Nord." The present writer has encountered specimens which seem to agree with Cooper's description and has been unable to find any previously described species of Gyrodactylus with which these individuals can be considered as identical.

During the summer of 1919, the writer was engaged in an investigation of the parasitic worms which infest fresh-water fishes at the Fairport Biological Station of the U. S. Bureau of Fisheries. In the course of routine examinations of fishes a number of instances of ectoparasitic trematode infestations were encountered. Under conditions of nature the opportunity for an ectoparasitic trematode outbreak to become serious is relatively small. On the other hand, when fishes are confined in ponds and pools in much denser population than occurs in nature, especially favorable conditions are given for development of heavy general infestation. Young fish in hatcheries thus have unusual opportunities for acquiring ectoparasitic trematodes, if such are present in the environment. Thereby, these parasites are transported to new localities by the distribution of the infested fishes.

The writer is deeply indebted to the members of the staff of the Fairport Station for aid in securing materials for study and is under especial obligation to Dr. H. S. Davis who first called his attention to the presence of ectoparasitic trematodes on the fishes which were being used in experimental work at the station. According to observations by Dr. Davis, trematodes have been fairly abundant on the gills of various species of fish from the cultural ponds and tanks at Fairport for a number of seasons.

Genus Gyrodactylus von Nordmann 1832

Small, spindle-shaped or cylindrical trematodes, usually less than 1 mm. long, living on the body surface or gills of fresh-water fishes. The two extremities of the body furnish characters for the separation of Gyrodactylus from other genera of ectoparasitic trematodes. In this genus the body terminates anteriorly in two small papillae while the posterior extremity is modified as an organ for attachment to the host. This organ of fixation (Figs. *a b*, text fig. 1) is a broadly expanded disc which bears two long, powerful hooks in the center and a series of 16 small spines arranged in a single row about its margin. The mouth occurs on the ventral surface some distance behind the anterior extremity. Eyespots, which are found in some genera of the family Gryodactylidae, are wanting in this genus. The eggs undergo development within the body of the parent and give rise to young individuals which are capable of infesting the fish without passing a larval existence in any intermediate host.

On August 20, 1919, the writer encountered an infestation by a member of this genus that seems to be significant. On the day preceding, about a dozen young catfishes, *Ameiurus melas* (Raf.) * had been removed from the Quarry Pond of the Fairport Station to a running water aquarium in the laboratory. In less than 24 hours after their removal to the laboratory a number of the fishes had died. A careful examination of the dead fishes failed to bring out any possible cause of death other than the presence of small trematodes of the genus Gyrodactylus scattered over the entire surface of the

Fig. 1.—*a*, *Gyrodactylus fairporti* sp. n., stained whole mount from ventral surface. Camera lucida drawing. x290. *b*, *G. fairporti*, posterior attachment disc slightly tilted ventrad and viewed from the side. x 830.

body. All of the fish in the aquarium were examined and found to be infected with this same parasite. After a preliminary study of the living worms the infected fish were placed in hot corrosive sublimate and the parasites preserved for later study. Thorough examination of these specimens has demonstrated that they belong to a species previously undescribed. To this new species the name *Gyrodactylus fairporti* is ascribed and the distinguishing characters are set forth in the following diagnosis.

* Mr. R. E. Richardson very kindly identified the host specimens.

Gyrodactylus fairporti new species

Gyrodactylus: In general form the central hooks of *G. fairporti* resemble those of *G. medius* Kath. (Cf. *a, b,* Text fig. 2). However, in *G. fairporti* these hooks are much simpler in form, are considerably longer, and throughout the length of the root-like portion are much less divergent than in *G. medius*. In *G. medius* the basal portions of these hooks approach each other at a single point near the place where the hooks bend ventrally to form the free, exposed region, while in *G. fairporti* the entire basal regions are almost parallel except at the proximal extremity and here occurs a slight bending toward the median line. The transverse clamps which serve as a means of articulation between the two hooks are much more prominent in *G. medius* than in *G. fairporti*. Wegener (1910) in giving the measurements of the

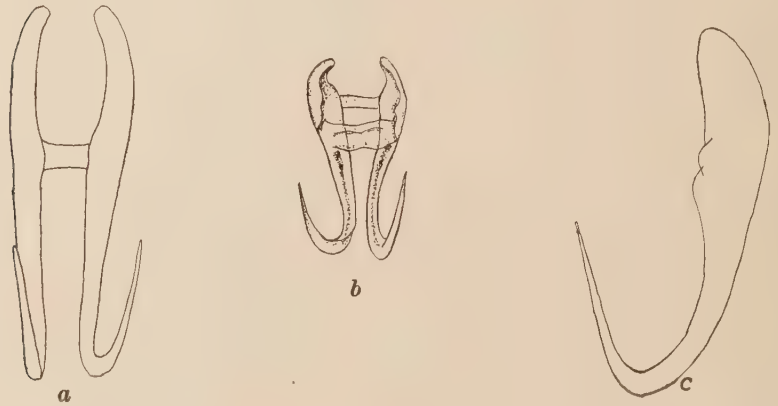

Fig. 2.—*a*, Ventral view of central hooks of *G. fairporti*, × 830. *b*, Central hooks of *Gyrodactylus medius* Kath. After Kathariner (1894, fig. 8). Magnification not in original; figure cannot be compared directly with *c*. *c*, Side view of central hook from *G. fairporti*. x 830.

central hooks of *G. medius* gave a range in length of from 37 to 40μ. In 50 individuals of *G. fairporti* the writer found the smallest hooks to be 53μ long and the longest 65μ. Hooks of the greatest number of individuals ranged between 59 and 62μ.

Other described species of Gyrodactylus present such distinctively different types of hooks that there is small chance of confusing any of them with either *G. medius* or *G. fairporti* if a careful examination of the hooks is made. For this reason no especial effort is made in this description to compare the hooks of *G. fairporti* with those of species other than *G. medius*. Attention should be called to the fact that the measurements of the hooks of *G. medius* Kath. are taken from the work of G. Wegener (1910) even though that author indicated some doubt as to the correctness of the determination of the materials

which he described under that name. The fact that the form with which he was dealing is one of the common species in central Europe and that his figures and descriptions agree so completely with those of the author of the species seem ample ground for assuming that his determination of the species was correct. The works of subsequent writers have failed to bring forth evidence of the presence of additional species of the genus in Europe closely resembling the species *G. medius*.

The small marginal spines of the posterior disc are very difficult to measure because of foreshortening due to the sloping margins of the disc and because of the fact that their proximal terminations are very indefinitely defined. In some individuals they were not more than 20μ long while in still others from the same host they were more than 30μ long.

Preserved specimens of *G. fairporti* are usually between 0.350 and 0.450 mm. in length. The maximum diameter of these same specimens varies from 0.050 to 0.075 mm. Practically all of the larger individuals carry an embryo in a fairly late stage of development (Fig. *a*, Text fig. 1).

Hosts: *Ameiurus melas* (Raf.) and *Cyprinus carpio* Linn. at Fairport, Iowa, from Quarry pond and culture ponds. Parasites attacking entire body surface of Ameiurus and appearing chiefly on gills of the carp.

Cotypes deposited in the U. S. National Museum, in the collection of the writer at Urbana, Illinois, and in the parasitological collection of the University of Illinois.

Genus Ancyrocephalus Creplin 1839

Throughout the months of July and August light infestations by an undetermined species of Ancyrocephalus were observed on the gills of various fishes from the ponds of the Biological Station. At the time these observations were being made this parasite was encountered most frequently and in greater numbers upon the gills of *Lepomis pallidus* though many other fishes were found to carry an infestation. Among other hosts observed at Fairport are *Ictalurus punctatus*, *Lepomis humilis*, and *Micropterus salmoides*. The gills of a specimen of *Esox lucius* from Lake Pokegama, Minnesota, displayed a heavier infestation than any encountered at Fairport. In preserved material the posterior extremity bearing the hooks and spines was so firmly embedded in the gill tissues of the host that details of structure essential for determination of the species were inaccessible.

On two other occasions members of the genus Ancyrocephalus have been reported from fresh water fishes of North America. Both of these earlier records resulted from work done on Canadian fishes.

J. Stafford (1905:681) recorded the occurrence of *Tetraonchus unguiculatus* Wag. (= *Ancyrocephalus paradoxus* Crepl.) from *Ambloplites rupestris* and *Eupomotis gibbosus*. He has given no figures or other data in confirmation of his determination. A. R. Cooper (1915:190) has recorded the occurrence of the same species on the gills of *Micropterus dolomieu* and identified other specimens from the same host species as *Ancyrocephalus cruciatus* (Wedl.). In both of these instances he has pointed out rather significant differences between his specimens and the descriptions of the species offered by European investigators. It is not improbable that further study would furnish additional points of difference between the North American and European representatives of this genus.

Worms of this genus do not seem to develop dangerous infestations such as are reported for the genus Gyrodactylus and are consequently of less importance economically.

TREATMENT OF INFESTED FISHES

Heavy infestations of Gyrodactylus may result in serious disturbances to the host. Unless relief is afforded death of the host is the usual outcome. Pratt, as already quoted, has mentioned the shriveled and functionless condition of the gills in a heavy infestation of rainbow trout by Gyrodactylus. Damage to the general surface of the body is not restricted to the direct damage due to piercing of the body covering by the hooks and spines. Inroads of bacteria, protozoa, and fungi are made possible by the lacerations produced by the hold-fast organs.

Fishes infested with Gyrodactylus are conspicuously inactive and weak. They allow the gill covers to remain much wider open than in the normal condition (Hofer, 1906:135). These, with other symptoms, European investigators have associated with Gyrodactylus infestation and to this pathological condition have applied the name Gyrodactyliasis or Gyrodactylus disease. Since the disease is apt to develop in aquaria and artificial enclosures rather than in the natural open waters, methods of direct treatment of the diseased fish are applicable. European workers have advocated a number of different methods of treatment for removing the parasites. All of these measures involve subjecting the parasitized fish to solutions of substances that are fatal to the parasites without harming the host. Conditions under which such treatment may be carried out vary so widely that wholesale operations of any sort are inadvisable until the individual method has been tested out. Temperature of the water and differences in the susceptibility of fishes of different species and different ages offer obstacles that make an empyrical formula for treatment not only unwise but also unsafe.

Hübner (1895: 191) has recommended a one-fourth of one per cent. solution of salicylic acid in which the infested fish are allowed to stand for one-half hour. Hofer (1906: 137) advocates this same treatment and in addition suggests another formula which he considers as of value, viz., one part of potassium permanganate to 100,000 parts of water.

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EXPERIMENTAL INGESTION OF THE OVA OF *FASCIOLOPSIS BUSKI;* ALSO THE INGESTION OF ADULT *FASCIOLOPSIS BUSKI* FOR THE PURPOSE OF ARTIFICIAL INFESTATION

Claude H. Barlow
Shoahsing, Chekiang Province, China

The experimental ingestion of the eggs of *Fasciolopsis buski* was carried out for the purpose of determining the time which would normally elapse after the eggs were swallowed till they would appear in the stools. It was desirable to find out how long a time is required for a patient to become ova-free in order to have some time-basis for the dismissal of patients from the hospital wards.

It was also desired to find the length of time it would take for ova to appear in the stool if an egg-laying adult fluke were swallowed and the length of time before ova from such a fluke might be expected to disappear from the stool if the fluke did not lodge in the intestinal tract as a parasite. The effect of the digestive fluids on ingested ova was incidentally noted.

The following tables show the results of the experiment. Ova, for the experiment, were obtained from the stools of patients infested with *Fasciolopsis buski* only. They were washed and swallowed in ordinary gelatine capsules.

November 10, 1918, at 11:30 a. m. many thousands of ova were swallowed. At 2:00 p. m. stool, not washed. In one slide, 1 *Fasciolopsis buski* ovum. In one slide 1 Trichocephalus trichiurus ovum. Fve slides negative.

November 11, 1918. Stool at 2:00. Large sample washed. Four slides negative. Three slides each with 1 *Fasciolopsis buski* ovum, in one of which was also 1 *Trich. trich.* ovum.

November 12. No stool.

November 13. Stool at 7:30 a.m. Whole stool washed. Eight slides negative. One slide with 3 *Trich. trich.* ova, and one with two such. Five slides with 1 *Fasc. buski* ovum each in two of which were 2 *Trich. trich.* ova each and in a third three such.

November 14. Stool at 7:00 a. m. Washed. Five slides negative. One slide had one *Fasc. buski* ovum, one had two and one four such. Two slides showed one *Trich. trich.* ovum in each.

November 15. Stool at 6:20 a. m. Washed. Six slides negative. Two slides with 1 *Fasc. buski* ovum in each, one of which had also 5 *Trich. trich.* ova. One slide showed 2 *Trich. trich.* ova.

November 16. Stool at 9:00 p. m. Washed. Five slides negative. One slide had 1 *Trich. trich.* ovum, two had two each, and two had four each.

November 17. No stool.

November 18. Stool at 7:30 a. m. Washed. Ten slides examined. All Fasciolopsis negative.

November 25. Slight diarrhoea. Stool Fasciolopsis negative.

The eggs appeared on the same or the following day that they were ingested and disappeared in eight days from the day of ingestion. In ward-patients who had taken an anthelmintic and were freed from flukes, no eggs appeared after two days if the anthelmintic had been followed by a large saline purge and, in most instances, did not persist after the first day. This, I believe, is due to the large size of the eggs, the smoothness of their shells, and to their weight. They settle rapidly in water. From the appearance of the eggs voided after ingestion, they received no hurt in the stomach from the action of the digestive fluids. No empty shells were seen in the stools.

FEEDING EXPERIMENT

February 15, 1920. Three live flukes, from a human patient, were covered with bread dough and offered to a pig. On smelling of the dough, the pig refused it, doubtless detecting the presence of the flukes. It was then forcibly fed and the stools examined for a few days till they became ova-free, which occurred at the end of seven days. The attempt to infect the pig was not repeated.

Fig. 1.—*Fasciolopsis buski* specimens traced in outline and swallowed at dates indicated: *a*, *b*, February 16, 1920; *c*, March 5, 1920; *d*, May 14, 1920; *e*, March 25, 1920; *f*, March 5, 1920; *g*, Fluke recovered alive, April 8, 1921. All figures one half natural size.

FIRST INGESTION EXPERIMENT

The care and attention incident upon the transportation of experimental animals from China to America making their use impracticable and it being imperative to have living eggs for the purpose of studying their development, the author undertook to infest himself by swallowing adult flukes obtained from Chinese patients by the use of anthelmentics.

February 16, 1920, at 5:30 p. m. two *Fasciolopsis buski* were traced and swallowed Fig. 1, *a*, *b*.

The first stool passed at 10:00 p. m. of the same day showed no signs of flukes or of ova.

February 17 at 11:30 p. m. the second stool examined showed no flukes and but two ova in ten slides.

February 18, at 9:30 p. m. the third stool examined showed no flukes and but one ovum.

February 19, at 4:30 p. m. the fourth stool examined showed no ova.

February 20, at 3:00 p. m. the fifth stool examined showed no ova.

None of the succeeding stools for a number of days showed any ova and the experiment was deemed unsuccessful. As the flukes

were swallowed on an empty stomach it is possible that they were digested before passing into the intestine and the following experiment would seem to corroborate this opinion.

SECOND INGESTION EXPERIMENT

March 5, 1920, at 11:50 a. m., three live *Fasciolopsis buski* were swallowed after administration of half a drachm of soda bicarbonate and were followed by one ounce of condensed milk. These flukes were taken just before eating lunch. They were recovered from a patient by the use of B-naphthol and when swallowed were of the color of Tallquist scale 95 per cent. All about the same size (Fig. 1, c.).

March 6, at 3:30 p. m. the first stool showed two ova in the first slide and five ova and one empty shell in the second slide. March 7, at 4:20 p. m. the second stool showed two ova in one slide. March 8, at 7:35 a. m. the third stool showed two ova to one slide. March 9, at 2:30 p. m. the fourth stool showed no ova in the first slide and only one in the second slide. March 10, at 7:00 a. m. the fifth stool showed one ovum in two slides. March 11, at 8:00 p. m. the sixth stool was not examined. March 12, at 10:00 a. m. the seventh stool showed one ovum in the first slide. March 22, at 1:00 p. m. the first slide showed five ova, the second 7 ova.

THIRD INGESTION EXPERIMENT

March 25, 1920. At 3:15 p. m., after a full meal, the six flukes were traced (Fig. 1, *e*) and swallowed. Their color viewed by transmitted light was about like Tallquist's haemaglobin scale 80 per cent., but by reflected light much darker than Tallquist 100 per cent.

March 26 to May 8 stools were examined and all showed ova. May 8, 1920, at 7:30 a. m. one slide showed three ova. This stool weighed 2,734 grains and contained 54 ova in 5 grains of stool. Estimated 29,500 ova in the whole stool. Stools were examined intermittently from the time of the second ingestion experiment and there was no time when they were not present in the stools, until the infestation was finally terminated.

FOURTH INGESTION EXPERIMENT

May 13, 1920, at 2:20 p. m., after a full meal, fifteen flukes were traced (Fig. 2, *a*) and swallowed in saline solution. In color they were about like those of the preceeding experiment. May 14, 1920, at 9 a. m., ten flukes were swallowed, all about the size of the traced fluke (Fig. 1, *d*). In all, thirty-six flukes were swallowed. The first two were unable to survive. The second ingestion was successful in establishing infestation. From March 6, 1920, until April 8, 1921, the stools were never free from ova. April 7, 1921, in order to rid himself of the infestation, the author took the following treatment:

At 6 p. m. magnesium sulphate oz. ½, fasting. April 8, 1921, at 7 a. m. B-naphthol gr. 30, taken fasting. At 7:30 a. m. B-naphthol gr. 20, taken fasting. At 8 a. m. B-naphthol gr. 20, taken fasting.

This was followed by one ounce of magnesium sulphate at 10 a. m. fasting. After free catharsis no flukes were passed so treatment was repeated as follows: B-naphthol gr. 20 at 1:30 p. m., gr. 20 at 2 p. m. and gr. 10 at 2:30, all taken fasting followed by magnesium sulphate oz. 1. After repeated stools, at 6:25 p. m., one live fluke was passed. It was nearly denuded of epithelium and spermatic fluid was oozing from the open terminals of the testes. It lived but a short time after being passed, but was traced alive April 8, 1921 (Fig. 2, *b*). It was 3 mm. thick and showed spots of epithelium still remaining.

April 9, no stool, magnesium sulphate ½ oz. was taken. April 10 to 18 all stools were washed and most carefully examined but no ova were found. Since that date a number of examinations have been made but no ova have been found. The last examination was made May 26, 1921.

Shreds of macerated flukes were searched for in all stools passed from April 8 to April 14, but there was nothing to indicate that there was present more than the one fluke, which was recovered. From the

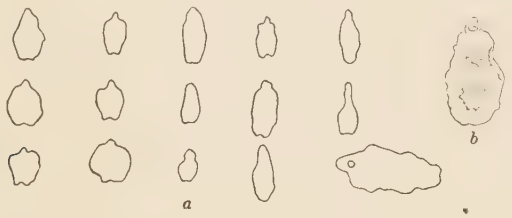

Fig. 2.—*Fasciolopsis buski* specimens swallowed as follows: *a*, May 13, 1920; *b*, April 8, 1921. Figures one half natural size.

above data it would seem reasonably certain that the fluke recovered was one of the three ingested March 5, 1920, as ova were constantly present from that date and twenty days elapsed between the date of their ingestion and the third ingestion experiment.

A comparison of the two flukes can be made from tracings of March 5, 1920 (Fig. 1, *f*) and April 8, 1921 (*g*). The outlines do not show the full extent of the difference in size as A is thinner than B, being only 2 as against 3 mm. thick and much more fully extended. March 31, 1921. Differential blood-count: Total leukocytes estimated, 8,000; L. Mononuclears 7.6; S. mononuclears, 23.0; polynuclears, 62.0; eosinophils, 6.3; basophils, 1.1. Morphological examination showed no extensive abnormalities. Platelets not increased.

It is interesting to note that some symptoms of a mild nature were observed by the patient, but that, considering the fact that he harbored but one fluke, these symptoms were probably psychological, though some mild edema was observed just prior to taking treatment. The swallowing of these flukes was indeed a nauseating process even

though the experimenter went into the dark-room to take them. They were put through a preliminary washing with alcohol and then washed thoroughly in saline solution.

All of the ova used in the study of the development of the eggs of *Fasciolopsis buski* were obtained from the one fluke which is evidence of the fact that cross-fertilization in these trematodes is not essential to the production of fertile ova. From a number of estimates made at various times from weighed stools, this one fluke passed from nine to twelve thousand ova daily.

So far as can be ascertained from literature at my command, there is no record of adult parasites being obtained by means of anthelmintics from one primary host and being fed to a different individual of the parasite's primary host with a resulting infestation of the second primary host.

CONCLUSIONS

Ingested ova appear in the normal stool as early as two and a half hours after being swallowed in gelatin capsules.

Patients become ova-free in from one to eight days without the use of cathartics and in from ten hours to two days after free catharsis.

After swallowing two live flukes which did not become parasitic, ova appeared in eighteen hours and disappeared in seventy-one hours.

Twenty-eight hours after swallowing three live flukes, one of which lodged and became parasitic, ova appeared in the stool and did not again disappear till a year later when an anthelmintic was taken and the adult fluke was expelled.

Some growth is noted in the fluke which stayed in but the growth is less than might be expected over a period of more than a year.

Eggs obtained from a single fluke infestation are fertile and have been hatched and the miracidium studied.

NOTE ON A CASE OF HUMAN INFECTION WITH *ISOSPORA HOMINIS* PROBABLY ORIGINATING IN THE UNITED STATES

FRANK G. HAUGHWOUT
Protozoölogist, Bureau of Science, Manila, P. I.

On the study of the feces of an American recently arrived in Manila from the United States, and who had been suffering from intermittent diarrhea and abdominal discomfort, the origin of which is not entirely clear, I have discovered a moderately heavy infection with *Isospora hominis* Rivolta, 1878 (emend. see Dobell, 1919). Circumstances connected with the case lead me strongly to the belief that the infection was contracted in the United States.

The object of this note is to call attention to this case and also to eleven others found in the United States since 1918, by Kofoid and his co-workers (1919, 1920) which seem so far to have escaped comment or even attention. In 1918 I directed attention (Haughwout, 1918) to the number of cases of human coccidiosis that were being detected in the Eastern Mediterranean area and in troops returned from there, and ventured the prediction that under the prevailing war conditions other cases might be expected to crop up at almost any time or place. It would seem as if this prediction is being fulfilled.

How serious a medical and sanitary problem is likely to be presented, it is hard to say. We have little knowledge of the clinical phenomena, and still less of the pathology of "human coccidiosis." The general impression is that the disease is not harmful to the adults who have been observed, but we have no knowledge of its effects on children or on people of lowered vitality. For that reason I think it unwise to adopt too placid an attitude toward these parasites.

Moreover, the outlook for an exceedingly interesting epidemiological study seems to me to be especially promising if the cases so far reported are traced and carefully studied, and all the contacts rounded up and examined. The cysts of the coccidia are highly resistant to untoward environmental conditions and to disinfectants and chemical reagents. They likewise seem to remain viable over long periods of time. We have reason to believe that the cysts of *Isospora hominis* will show the same attributes. This makes the prevention of dissemination a much more serious problem than is the case with cysts of the other intestinal protozoa of man, which are much less resistant to untoward conditions and are embued with far less powers of longevity.

With the idea of aiding in any studies that may be undertaken in the United States, Professor Elmer D. Merrill, director of the Bureau of Science, has authorized the transmission of material from our case to the following specialists in the United States, where it will be on hand for comparison:

Professors Gary N. Calkins, Columbia University, New York; R. W. Hegner, Johns Hopkins University, Baltimore, Md.; Henry B. Ward, University of Illinois, Urbana, Ill.; Charles A. Kofoid, University of California, Berkeley, Calif.; R. B. Gibson, Iowa State University, Iowa City, Ia.; Ernest E. Tyzzer, Harvard University Medical School, Boston, Mass.; Kenneth M. Lynch, Medical College of the State of South Carolina, Charleston, S. C.; James C. Todd, University of Colorado, Boulder, Colo.; Mark F. Boyd, University of Texas, Galveston, Tex., and Allen J. Smith, University of Pennsylvania.

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Kofoid, Charles A., Kornhauser, Sidney I., and Plate, J. T. 1919.—Intestinal Parasites in Overseas and Home Service Troops of the U. S. Army. Jour. Amer. Med. Assoc., 72:1721.

Kofoid, Charles Atwood and Swezy, Olive. 1920.—On the prevalence of Carriers of Endamoeba dysenteriae among Soldiers Returned from Overseas Service. New Orleans Med. Surg. Jour., 73:4-11.

BOOK REVIEW

SANITARY ENTOMOLOGY. The Entomology of Disease, Hygiene and Sanitation. Edited by William Dwight Pierce, Ph.D., Boston, Richard G. Badger, The Gorham Press, 1921, pp. xxvi + 19-518; plates, 28; text-figures, 88.

Although a considerable number of reference works relating to the sciences of Sanitation, Medical Entomology and Parasitology and their interrelations are now available, the present volume is of such importance that it will be welcomed to the library of all interested persons. As indicated by the editor, the work is the final outgrowth from a series of lectures delivered by a group of specialists in Washington in 1918 to a class of entomologists who were preparing themselves for service during the war period. These mimeographed lectures have now been completely revised to include the entomological problems of peace times as well as those presented by the emergency of war. The volume, written by Dr. Pierce and nine collaborators, contains thirty-five chapters, of which eleven are of especial interest in the study of parasitology and its relations to entomology. The general plan of the work is the treatment of the various insect and other Arthropodan groups in their systematic sequence from the highest to the lowest, a chapter being devoted to the diseases transmitted by each group, followed by chapters on biology and control. In this manner the following groups of Arthropods are discussed:

The non-biting flies, with special chapters on the house fly and the Dipterous groups causing myiasis.

The blood-sucking flies (some aberrant species of which are treated in the discussion of myiasis), with special chapters on

Horse flies and mosquitoes

Lice and louse-borne diseases

Diseases carried by fleas

Diseases transmitted by cockroaches

The bedbug and other bloodsucking bugs

Diseases caused or carried by mites and ticks.

In the chapters on the diseases carried or transmitted by the various groups of insects, the causative organisms are treated uniformly beginning with the plant forms, followed by the Protozoa, Platyhelminths and Nemathelminthes. Each chapter is terminated by a short bibliography or list of references cited.

A chapter on the *Relation of Insects to the Parasitic Worms of Vertebrates,* by Brayton H. Ransom, is of especial interest. In this chapter, the various subjects, such as the mode of infection of the insect and the vertebrate hosts and the species of worms found in insects, are discussed in considerable detail. This chapter includes a compendium of the insects with their known worm parasites, a feature that will be of great value to teachers and students. The final chapter of the work, *Tabulation of Diseases and Insect Transmission,* has twenty-four pages devoted to a summary of the various diseases discussed in the remainder of the book, which are here listed alphabetically together with their causative organism, insect transmitter, the method of transmission and the rôle of the insect concerned, whether a parasite, intermediate host, mechanical carrier or what-not. This feature of the volume will also be of considerable value for reference purposes.

The style of type used is in the main excellent although there is valid objection to the use of large capitals in the text to designate the common names of diseases and other terms. The price of the volume ($10.00) must be considered as being distinctly exorbitant even in these days of high prices. But there can be no doubt that this volume will fill a most important place as a text-book or reference work.

NEW HUMAN PARASITES

Trypanopsis malignus Leger, 1920.—A small number of parasitic protozoa with or without flagella were found in smears from the liver of a European patient who died after 11 years' residence in French Guiana following a fever of unknown etiology. These flagellates resemble somewhat the forms known as *Herpetomonas brasiliensis* (Franchini, 1913) described from man in Brazil which although living in the blood of man show the morphological characteristics and even the encystment observed in the intestinal flagellates of insects. *T. malignus* differs radically from Franchini's species, however, in the complete absence of pigment and of cysts. It shows slight resemblances to trypanosomes of the type of *Trypanosoma lewisi* but appears not to be a true trypanosome (Ann. Inst. Pasteur, 34:481-496, pl. 16).

Spirochaeta acuta Séguin, 1920.—This buccal spirochete with flattened spirals and pointed ends grows in association with a fusiform bacillus in a culture medium composed of equal parts of gelose (Veillon) and ascitic fluid at a temperature of 37°C. It is intimately dependent upon the bacterium, but can be grown in pure culture if separated from the bacterial growth by a collodion membrane, showing that the substance produced by the bacterium which are essential to the growth of the spirochete will traverse collodion membranes. (Compt. rend. Acad. sci., 171:1243-1244.)

Gongylonema hominis sp. dub. Stiles, 1921.—This name is suggested on purely practical grounds for the worm described and figured by Ward (1916) as *Gongylonema* (?) *pulchrum* in order to avoid erroneous deductions as to life history until such time as sufficient material from man becomes available to establish the specific characters. A third case of probable Gongylonema infestation of man is recorded (Georgia), the two previous and more certain cases having been recorded from Arkansas (Ward) and Florida (Stiles, 1917). (Pub. Health Rep., 36:1177-1178.)

Entamoeba paradysenteria Chatterjee, 1920.—This amoeba which was found in a case of fatal dysentery in Calcutta is stated to differ from *Entamoeba histolytica* in that the nucleus is massive, not karysomic, there is a marked distinction between ectoplasm and endoplasm, and chromidia are absent. Furthermore not only the large intestine but the small intestine was attacked, with peritoneal involvement. The new species according to the author differs also from both *Entamoeba nana* and *Vahlkampfia limax* in important morphological details as well as in its pathogenicity (Philippine J. Sci., 17:385-394, 3 pl.).

Councilmania lafleuri Kofoid and Swezey, 1921.—A sixth species of amoeba from the human intestine representing a distinct genus is described from material derived from 10 cases under observation for varying periods. This amoeba may be present in enormous numbers, appears to have pathogenic capacities, and has apparently been passed over as *Endamoeba coli* because of its large size, its eight-nucleated cyst, and its high resistance to stains. It differs from *E. coli* in the following particulars: Free stage very active, pseudopodia thrust out suddenly, ectoplasm sharply separated from endoplasm; red blood corpuscles ingested readily; peripheral chromatin of nucleus in a thin layer, karyosome large (and excentric with halo as in *E. coli*) or often seen in premitotic condition with chromatin dispersed in granules in a sphere, ring, or skein, without halo and often central. Encysted stage with very thick cyst wall; spheroidal, ellipsoidal or asymmetrical (less often spherical as generally the case in *E. coli*); less readily stained; glycogen body more resistant to iodine; nuclei with little peripheral chromatin and large, generally central or but slightly excentric, dispersed karyosome; chromatoidal bodies less acicular in early stages, fasciculate, massed centrally in later stages and contributing to chromophile buds; chromophile ridge forms a bud through a pore in the cyst wall, which detaches uninucleate amoebulae. (Univ. Calif. Publ. Zool., 20:169-198, 5 pl.)